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CONTENTS

THE NATURE OF THE HETEROPHILIC ANTIBODIES IN INFECTIOUS MONONUCLEOSIS. I*

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After the occurrence of an increased titer of agglutinins and of hemolysins for erythrocytes of sheep in infectious mononucleosis became firmly established, three problems presented themselves for investigation: (*a*) The nature of the antibodies, (*b*) Their relation to the antibodies that are found in serum disease and (*c*) Separation from true infectious mononucleosis of cases that resemble it clinically and hematologically, and sometimes even serologically.

From the fact that the so-called Forssman heterophilic antigen is known to produce in the rabbit and in man hemolysins and agglutinins against the erythrocytes of the sheep and that the antibodies found in serum disease were interpreted as being of the Forssman heterophilic type, it was assumed by most of the recent writers on the serological aspects of infectious mononucleosis that the antibodies in the latter disease are also of the Forssman heterophilic type.   To a student of the heterophilic immunological reactions, it is apparent that the term "heterophilic antigens and antibodies" is not synonymous with the term "Forssman antigens and antibodies," that the latter represent merely a special subgroup within the large number of the heterophilic complexes. Heterophilic antibodies could be defined as antibodies that have the ability to react with antigens that are apparently entirely unrelated to those that stimulated their production.   Heterophilic antigens could be defined, from the above, as antigens capable of stimulating the production of such antibodies.   The Forssman antibodies represent the special case of heterophilic

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antibodies that react with the erythrocytes of the sheep on the one hand, and with the heterophilic antigen of the guinea pig tissues on the other. There are other sub-groups of heterophilic antigens and antibodies, for example, antibodies that react with human erythrocytes and are produced in the goat by the inoculation of dysentery bacilli.

It was suggested recently[4] that it may help to clarify the confusion if the term "Forssman complex" be used for the antigenic substance contained in the tissues of the guinea pig and of the other animals known to belong to the guinea pig group which, when injected into a host like a rabbit or into another species belonging to the rabbit group, produce antibodies against erythrocytes of sheep. The other complex that was mentioned, the one consisting of dysentery bacilli, of human red blood cells, and of the host, the goat, could be referred to by the name of its discoverer as the "Eisler complex." For further discussion the reader is referred to an earlier paper.[2]

It is very interesting that the solution of the problem of the nature of the heterophilic antibodies in infectious mononucleosis was attempted simultaneously, and apparently independently of each other, in three different laboratories. C. A. Stuart and associates[5,6] noted that while the agglutinins for erythrocytes of sheep were readily removed by raw and boiled erythrocytes of sheep, they were only very slightly absorbed by different tissues of the guinea pig. They concluded that the antibodies, while heterophilic in nature, are not of the Forssman heterophilic type. Bailey and Raffel[1] treated the serums of patients with infectious mononucleosis with a large variety of antigens and found that, of the known carriers of the Forssman heterophilic antigen, only the erythrocytes of sheep removed the agglutinins for this animal's erythrocytes thoroughly, that the kidney of the horse and some strains of Cl. Welchii did it less effectively, and that the tissues of the guinea pig and of other members of the guinea pig group failed to do it altogether. On the other hand, boiled erythrocytes of the ox showed a very high affinity for the heterophilic antibodies in the serum of patients with infectious mononucleosis. They concluded that the heterophilic antibodies in infectious mononucleosis are not of the heterophilic or Forssman type.

This study includes: (a) eleven cases of horse serum disease due to injections of plain horse serum, of diphtheria antitoxin, of antimeningococcic serum, and of scarlet fever streptococcus antitoxin; (b) seven cases of infectious mononucleosis; (c) two borderline cases which resembled infectious mononucleosis clinically and hematologically; and (d) a series of normal controls with a negative history as to infectious mononucleosis and serum injections.

Our studies of the relation of the erythrocytes of the ox to the antibodies in infectious mononucleosis and in serum disease will be reported separately.

TECHNIC OF ABSORPTIONS

The serums were inactivated for 30 minutes at 56°C. They were absorbed one or more times with suspensions of the kidneys of the guinea pig, the rabbit, and the horse, and with the erythrocytes of the sheep The kidneys were kept frozen in the refrigerator until needed. They were then permitted to thaw out and were washed repeatedly in a physiological solution of sodium chloride until the washings were free of blood. They were mashed into a fine pulp and were used for adsorption as a 20 per cent suspension. The erythrocytes of the sheep were washed three times, packed well, and used for adsorption as a 20 per cent suspension.

To a volume of undiluted inactivated serum one and one-half volumes of the tissue or of the suspension of erythrocytes were added. The mixtures were kept at room temperature for 1 hour and were shaken vigorously at intervals of 15 minutes. They were then centrifuged, and the clear supernatant serum was transferred to other test tubes. A quantity of the serum sufficient for the various titrations was removed, and a second absorption was carried out by treating the balance of the serum with an equal amount of the suspensions. The further absorptions were carried out in a similar manner. The effect of the repeated absorptions upon the dilution of the serum was taken into consideration.

TECHNIC OF TITRATIONS

Hemolysins and agglutinins for the erythrocytes of the sheep were studied before and after absorption. The titers of the hemolysins following absorption were occasionally difficult to determine, due to interference by the anticomplementary effect of the tissue suspensions. However, the results were parallel to those obtained for the agglutinins. For the sake of simplicity only the latter are tabulated.

Serum dilutions varying from 1:2.5 were set up for the test and 0.1 cc. of a 2 per cent suspension of washed sheep cells was added. The tubes were left at room temperature for one hour and then in the ice-box overnight. The test tubes were shaken vigorously until the sediment of the cells became suspended.

The reading was done with the low power of the microscope (32 mm. objective). The titers as they are recorded in the tables represent the so-called one plus reading, and signify distinctly microscopically discernible clumping. (For details of technic see Davidsohn.[4])

C. A. Stuart and associates[6] recently emphasized that the differences in the dilutions of serum that are recorded in the publications of various authors make it very difficult and even impossible to compare titers with each other.   Most of these

TABLE 1

TABLE OF DILUTIONS

TEST TUBE	SERUM DILUTION	FINAL DILUTION OF SERUM	AMOUNT OF SERUM
		cc.	cc.
1	1:2.5	1:3.5	.1
2	1:5	1:7	.05
3	1:10	1:14	.025
4	1:20	1:28	.0125
5	1:40	1:56	.00625
6	1:80	1:112	.003125
7	1:160	1:224	.001563
8	1:320	1:448	.000781
9	1:640	1:896	.000390
10	1:1280	1:1792	.000195
11	1:2560	1:3584	.000098
12	1:5120	1:7168	.000049
13	1:10240	1:14336	.000024
14	1:20480	1:28672	.000012
15	1:40960	1:57344	.000006
16	1:81920	1:114688	.000003

difficulties could be eliminated by reporting titers in terms of final dilutions.   That would make the results of various writers more easily comparable.   We heartily endorse the suggestion of Stuart and associates, and we followed it in this paper.

The second column of table 1 shows the dilution of the serum before the addition of other reagents (erythrocytes, et cetera). That dilution is commonly reported as the titer.   The third column expresses the true or final dilution of the serum, after the addition of the other reagents.   The titers in this paper are expressed in terms of the final dilution.   The table will enable

those who are interested to convert the titers of the previous publications on this subject by Davidsohn.[3,4] The final column gives the actual amounts of the serum in each test tube.

Table 2 shows the results of the absorption of six serums of normal controls. They were especially selected among those with higher titers of agglutinins for the erythrocytes of the sheep. The effect of absorption is estimated in terms of percentages of absorbed antibodies. If the antibodies were left intact, the percentage of absorption equals zero; if they were removed completely, the absorption equals 100 per cent, with all gradations

TABLE 2

Blood Serum of Normal Individuals

CASE NUMBER	TITER BEFORE ABSORPTION	PERCENTAGE OF ABSORPTION OF AGGLUTININS FOR THE ERYTHROCYTES OF THE SHEEP			
		After first absorption with			
		Erythrocytes of sheep	Kidney of		
			Guinea pig	Rabbit	Horse
1930	14	50	100	50	100
1936	28	100	100	75	100
2029	14	100	100	100	100
2101	28	100	100	50	100
2122	14	100	100	0	100
2256	14	100	100	0	100
Average.....	19	92	100	46	100

between these two extremes. The table shows at the bottom the averages of the preliminary titers and of the percentages of the absorbed antibody. The result of the treatment with the kidney of the rabbit shows the effect of the nonspecific absorption and should be subtracted in the evaluation of the effects of absorption by the other antigens. The effect of the nonspecific absorption by the rabbit tissue varied from zero to 100 per cent, and its average was 46 per cent. The two other antigens were about equal to each other in their effect. That finding is in agreement with the well established Forssman heterophilic nature of the natural antibodies against sheep cells in the normal serums.

Table 3 shows the effect of absorption of the serum from patients with horse serum disease.

Due to insufficient quantities of serum in some of the cases, not all absorptions could be carried out with each serum. The absorption of the serums of patients with serum disease shows a similar effect of the kidneys of the horse and the guinea pig, and of the sheep cells, as in the case of normal serums. The differences are accounted for by the higher titers, as evidenced in case

TABLE 3

BLOOD SERUM OF PATIENTS WITH SERUM DISEASE

CASE NUMBER	TITER BEFORE ABSORPTION	PERCENTAGE OF ABSORPTION OF AGGLUTININS FOR THE ERYTHROCYTES OF THE SHEEP											
		After first absorption with				After second reabsorption with				After third reabsorption with			
		Erythrocytes of sheep	Kidney of			Erythrocytes of sheep	Kidney of			Erythrocytes of sheep	Kidney of		
			Guinea pig	Rabbit	Horse		Guinea pig	Rabbit	Horse		Guinea pig	Rabbit	Horse
27	448	92	92			100	100						
28	896	100	98				99						
33	448	92	94			100	98						
34	3584	98	99	94	97	100	99.6	98	99			98	100
36	1792	94	94	75	94	98	97	87.5	98	100	98	94	100
4	448	94	98	87.5	87.5	97	100	87.5	97	100		94	100
12	112	95	100	0	97	87.5		50	100	100		50	
13	112	100	100	50	97			75	100			75	
21	224	97	94	75	87.5	100	100	87.5	94			100	100
7	56	100	100	50	87.5			75	100			100	
1	28	100	100	50	100			50				100	
Average.	741	97	97	60	93	98	99	76	98	100	98	89	100

1, where the absorptions were as complete as in the normal serums, and in cases 7, 12, and 13, where the effect of the absorption was approximately complete in spite of the moderately elevated titers. The effect of the absorption with the kidney of the rabbit is more pronounced than in normal serum.

Table 4 shows striking differences in the effect of the absorption by the various tissues. The nonspecific absorption by the kidney of the rabbit is less marked than in the cases with serum

disease. The striking finding is the obvious failure of the kidney of the guinea pig to absorb specifically the agglutinins for the sheep cells. The differences between the effect of the absorption by the kidney of the rabbit and the guinea pig kidney, which is considered the classical carrier of the Forssman antigen, are negligible and within the range of experimental error. This confirms the findings of Stuart and associates and of Bailey and Raffel. The ability of the kidney of the horse to absorb the agglutinins for sheep cells was close to that of the sheep cells in the first absorption and became equal to it after the second absorption. That result is somewhat in variance with the report of Bailey and Raffel because it shows that when a sufficient number of absorptions is carried out, the effect of the absorption with the kidney of the horse becomes equally as complete as that with the sheep cells. The differences are fully explained by variations in the avidity of absorption. The result conclusively establishes that the antibodies in infectious mononucleosis are heterophilic in nature but that they are not of the Forssman type.

Table 5 brings out the differences between cases with infectious mononucleosis on the one hand, and normal controls and those with serum disease on the other. Particularly instructive is the behavior of cases 36 and 11, because both had the same titers.

The differences are further emphasized in table 6 which compares the averages obtained from the second, third and fourth tables.

Table 7 presents the results of absorptions in two cases with the clinical picture somewhat resembling infectious mononucleosis, and with blood findings very similar to those commonly seen in that disease. In the first case the titer of agglutinins for erythrocytes of sheep was below the level which we consider as indicative of infectious mononucleosis, and that diagnosis could be eliminated on that account, but the titer in the second case was of a level which we consider as borderline.[3,4] The absorption with the kidney of the guinea pig eliminated the diagnosis of infectious mononucleosis without any doubt. We believe that such an absorption test may help to exclude infectious mononucleosis in similar and not at all rare cases.

TABLE 4

Blood Serum of Patients with Infectious Mononucleosis

PERCENTAGE OF ABSORPTION OF AGGLUTININS FOR ERYTHROCYTES OF THE SHEEP

| CASE NUMBER | TITER BEFORE ABSORPTION | After first absorption with | | | | After second re-absorption with | | | | After third re-absorption with | | | | After fourth re-absorption with | | | | After fifth re-absorption with | | | |
| | | Erythrocytes of sheep | Kidney of | | | Erythrocytes of sheep | Kidney of | | | Erythrocytes of sheep | Kidney of | | | Erythrocytes of sheep | Kidney of | | | Erythrocytes of sheep | Kidney of | | |
			Guinea pig	Rabbit	Horse		Guinea pig	Rabbit	Horse		Guinea pig	Rabbit	Horse		Guinea pig	Rabbit	Horse		Guinea pig	Rabbit	Horse
1	896	97	75	50	87.5	96	75	75	87.5	100	75	75	100		87.5	75					
6	896	97	0	50	50	97	50	0	87.5	100	50	0	100		50	50					
7	7168	99.6	75	75		100	75	87.5			87.5	87.5			94	94			94	87.5	
7	3584	99.6	87.5	87.5	87.5	99.6	87.5	87.5	97	100	87.5	87.5	100								
7	448	100	50	50			50	50			75	50									
9	1792	97	75	87.5		100	75	75	100	100	75	75			50	50			75	75	
10	224	94	50	50	97	100	75	75			75	75									
11	1792	94	75	75	87.5	97	75	50	87.5	97	87.5	75	97	100	94	94	100		97	87.5	
11	896	87.5	0	0	50	94	50	50	87.5		50	50	94		75	75	100		87.5	75	
12	1792	92	75	75	97	100	87.5	87.5	100	100	94	94		100	97	97			100	100	
Average	1949	96	56	60	80	98	70	64	92	99	76	67	98	100	78	76	100		91	85	

TABLE 5

Comparison of Titers

PERCENTAGE OF ABSORPTION OF AGGLUTININS FOR THE ERYTHROCYTES OF THE SHEEP

(Kidney sub-columns = Guinea pig, Rabbit, Horse)

CASE NUMBER	TITER BEFORE ABSORPTION	After first absorption with				After second re-absorption with				After third re-absorption with				After fourth re-absorption with				After fifth re-absorption with		
		Erythr. of sheep	Guinea pig	Rabbit	Horse	Erythr. of sheep	Guinea pig	Rabbit	Horse	Erythr. of sheep	Guinea pig	Rabbit	Horse	Erythr. of sheep	Guinea pig	Rabbit	Horse	Guinea pig	Rabbit	Horse
Normal controls																				
1936	28	100	100	75	100	100														
Serum disease																				
12	112	75	100	0	94	87.5			94	100			98							
36	1792	94	94	75	94	98	97		94	100	98	94	98							
Infectious mononucleosis																				
7	448	100	50	50	87.5	50	75	50	97	97	75	50	100	100	50	50	100	75	75	
11	1792	94	75	75	87.5	97	97	75	87.5	97	87.5	75	97	100	94	94	100	94	87.5	

TABLE 6

Averages of Titers and of Percentages of Absorption of Agglutinins for Erythrocytes of the Sheep

TITER BEFORE ABSORPTION	After first absorption with				After second re-absorption with				After third re-absorption with				After fourth re-absorption with				After fifth re-absorption with		
	Erythr. of sheep	Guinea pig	Rabbit	Horse	Erythr. of sheep	Guinea pig	Rabbit	Horse	Erythr. of sheep	Guinea pig	Rabbit	Horse	Erythr. of sheep	Guinea pig	Rabbit	Horse	Guinea pig	Rabbit	Horse
Normal controls																			
19	92	100	46	100	92	99	50	100											
Serum disease																			
741	97	97	60	93	98	99	76	98	99	98	89	98	100	78	76	98			
Infectious mononucleosis																			
1949	96	56	60	80	98	70	64	92	99	76	67	98	100	78	76	100	91	85	

Our results, together with those of Bailey, demonstrate for the first time the existence of a common heterophilic antigen which is not identical with the Forssman antigen, in the erythrocytes of the sheep and in the kidney of the horse.   This antigen reacts with varying degrees of avidity with the heterophilic antibodies in the serum of patients with infectious mononucleosis.

TABLE 7

BLOOD SERUM OF PATIENTS WITH SYMPTOMS AND A BLOOD PICTURE RESEMBLING INFECTIOUS MONONUCLEOSIS

CASE NUMBER	TITER BEFORE ABSORPTION	PERCENTAGE OF ABSORPTION OF AGGLUTININS FOR ERYTHROCYTES OF THE SHEEP											
		After first absorption with				After second reabsorption with				After third reabsorption with			
		Erythrocytes of sheep	Kidney of			Erythrocytes of sheep	Kidney of			Erythrocytes of sheep	Kidney of		
			Guinea pig	Rabbit	Horse		Guinea pig	Rabbit	Horse		Guinea pig	Rabbit	Horse
1	56		100	50	100			50				50	
2	112	100	100	100	100								

CONCLUSIONS

1. The antibodies (agglutinins and hemolysins for sheep cells) in infectious mononucleosis are heterophilic in nature, but are not of the Forssman heterophilic type.

2. The erythrocytes of the sheep and the kidney of the horse contain at least two common heterophilic antigens: (a) The Forssman heterophilic antigen; (b) the heterophilic antigen that reacts with the heterophilic antibodies found in infectious mononucleosis.

3. The absorption of a specimen of serum with the kidney of the guinea pig and of another sample of the same serum with the kidney of a rabbit, offers a convenient method for the differentiation of infectious mononucleosis from serum disease, and for the diagnosis of borderline cases.   The agglutinins for sheep cells will be almost completely absorbed by the kidney of the guinea pig but only partly by the kidney of the rabbit in cases of serum disease and in cases that are not infectious mononucleosis, while

in infectious mononucleosis the absorption with both tissues will be only partial and about equal in degree.

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TOXICOLOGY IN CHILDREN*

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Accidental poisoning in children by various household articles is by no means rare. Such poisonings generally arise from the innocent ingestion of these substances as a result of children playing with them. The accidental poisoning of children and infants occurring in hospitals is usually due to an error in medication brought about by negligence in not reading the label, or by using materials from unlabelled containers. Among the sources of poisons may be mentioned the silver polishes containing cyanide salts; various dry cleaning fluids composed of carbon tetrachloride, trichlorethylene, pentachlorethane, propylene chloride, gasolene containing tetra-ethyl lead, benzene, and other solvents; many deodorants, germicides and antiseptics containing carbolic acid, lysol, cresol, bleaching powder, potassium chlorate, bichloride of mercury, iodine, boric acid, oxalic acid, and sodium carbonate (washing soda). Insecticides and poisons for rodents, in the form of powder, solution or paste and containing strychnine, Paris green, phosphorus, white arsenic, or sodium fluoride, have been the cause of many incidences of poisoning. Ordinary household drugs, especially sugar coated cathartic pills, containing phenolphthalein, strychnine or atropine in some form and, left carelessly about the house, cause fatal poisoning much more often than is generally known. Poisoning from shoe polish or shoe dyes containing benzene or nitro-benzene are occurring more frequently. Chronic poisoning by carbon monoxide, due to leaking gas pipes or stoves in the house, or due to the inhalation of exhaust gases while playing around motor cars often goes

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unrecognized. Living in freshly varnished and non-ventilated rooms is dangerous, because of the evaporation of the turpentine or wood alcohol from the varnish. Children scraping and biting the lead-containing paint on toys, bed-steads, and other furniture, is a common form of poisoning. Ethyl (grain) alcohol and whiskey have occasionally caused poisoning.

The following cases culled from the files of the Chief Medical Examiner of the City of New York give some conception as to the nature of these incidences. Errors in the administration of therapeutic products or lack of knowledge on the part of nurses and other attendants has been responsible for a number of these deaths.

Acute cases in children are generally irritating poisons affecting the gastro-intestinal tract which one tries to eliminate with lavage and hypodermoclysis. In chronic cases the poison generally has penetrated the body and efforts must be made to remove it. This may be difficult at times, chiefly because these poisons are not always easy to detect due to the minute amounts present or because they are eliminated intermittently. The urine, feces and blood should be chemically analyzed in the latter cases.

BORIC ACID POISONING

Three infants were each given a hypodermoclysis of 150 cc. of saturated boric acid solution instead of a physiological saline solution. It was estimated that each received between 5.5 and 7 grams of boric acid, whereas the average adult fatal dose is 3 to 5 grams. Soon after the administration of this solution, the infants began to cry out and writhe in pain. Salivation, vomiting, diarrhea, bloody urine and delirium followed in rapid order. The skin became cyanotic, cold and clammy and death followed within three and one-half to four hours. At necropsy, there was some superficial cutaneous desquamation with characteristic edema of the bladder and submucosal hemorrhage. Otherwise there was only a generalized visceral congestion. Chemical analysis revealed 56 mgm. boric acid in 100 grams of composite sample of brain, liver and kidneys.

Detection

The specimen of urine, stomach washing or finely divided tissue is made strongly alkaline with a solution of sodium hydroxide or sodium carbonate and evaporated to dryness on the water bath. It is then carefully ashed at dull red heat. The ash is

acidified with concentrated HCl, and divided into two portions. To one portion ethyl alcohol is added, and then ignited.   A green flame indicates boron.   This flame examined with the spectroscope gives four characteristic absorption bands in the yellow, green, and blue green.   The second portion is diluted with a little water and tested with turmeric paper.   If boric acid is present the paper turns a red brown, which is intensified on drying.   In acute poisoning by mouth it is possible to determine whether boric acid or borax was taken, by analyzing the stomach contents or stomach washing.   The internal organs and the urine are useless for this purpose.   If the stomach washing is very alkaline it indicates borax; further, if the stomach contents are first dried on the water bath, ethyl alcohol will extract boric acid, but not borax.   If it is desired to estimate the amount of boron present, the material made alkaline with Na_2CO_3 is dried and ashed at low red heat.   The ash is then thoroughly extracted with dilute, hot hydrochloric acid, transferred to a volumetric flask and water added to a definite volume.   To 10 cc. of this solution is added some turmeric solution and the color that is produced is compared with that of a standard boric acid solution similarly colorized with turmeric solution.

Treatment

Symptomatic and supportive treatment was immediately instituted in these cases, but to no avail.   It must be stated, however, that the mode by which this poison gained entrance into the body in these cases is extremely rare.   Most cases result from accidental ingestion of boric acid or borax, and in such cases the treatment should be directed to removing the poison by gastric lavage and colonic irrigation, to accelerate excretion by giving fluids, chiefly in the form of alkaline drinks, and by intravenous calcium gluconate administration.   Supportive measures and symptomatic treatment to combat collapse, et cetera, must be instituted.   In order to avoid errors, hospitals as a general rule, color their boric acid solutions with eosin or some other non-toxic dye.

In this connection, it is of importance to call attention to a

form of chronic boric acid poisoning which is generally not recognized. It can only be detected by chemical analysis of the urine and feces. It occurs chiefly in infants, resulting from unwashed nipples which have been kept in boric acid solution, or from an old custom of giving babies cloths to suck which have been saturated in boric acid solutions or in which boric acid has been wrapped for the purpose of clearing up "white mouth" or thrush. The continued use of borated glycerine suppositories has led to chronic poisoning only. The symptoms in such cases are similar to those of the acute form, but much milder. The infants frequently are very fretful, apparently have considerable abdominal pain, may have an irritating dermatitis, show anorexia, and frequent periods of hematuria.

SODIUM CARBONATE (WASHING SODA) POISONING

A hospital nurse made up the feeding formulas for her charges, taking the lactose from a tin container, as usual. Shortly after the babies were fed, they became violently ill, with vomiting, diarrhea, tetanic contractions, collapse, coma and three of the patients died. At necropsy, the rugae of the stomach were well defined, intensely congested presenting a few hemorrhagic spots. The mucosa was pale and presented a glazed appearance. Bloody curds with a strongly alkaline reaction were found in the gastro-intestinal tract. The rest of the viscera showed intense passive congestion. The blood showed an alkali reserve of 85 per cent. The gastric washings gave an intensely alkaline reaction.

The white powder in the tin container, labelled lactose was examined and found to contain 28 per cent lactose and the remainder was sodium carbonate. Investigation failed to reveal how the soda got into the milk sugar.

Detection

The stomach contents, or the stomach washings (with water) must be used in analyzing for sodium carbonate. Due to the presence of Na^+ and CO_3^{--} ions in the blood and tissues normally, and also due to the buffer action of the blood and tissue fluids, it is impossible to definitely establish whether any exogenous sodium carbonate is present in the blood, tissues or urine. The stomach washings can be tested directly, unless a large amount of food is present. If so, the stomach contents should be dialyzed, and the resulting dialyzate used for the tests. A strip of litmus paper is introduced in order to ascertain whether the

material is at all alkaline.   If so, a few drops of phenolphthalein solution are added to some of the material.   A deep red color is produced if sodium carbonate or sodium hydroxide is present. Sodium bicarbonate will give only a faint pink.   To differentiate between sodium hydroxide and sodium carbonate, hydrochloric acid is added to some of the material.   The liberation of much gas (CO_2) indicates carbonate.   For the quantitative estimation, a known excess of N/20 sulfuric acid is added to a measured amount of the diluted and filtered stomach contents, then boiled one to two minutes, cooled, and the sulfuric acid remaining is titrated with N/20 NaOH.   From the amount of sulfuric acid neutralized by the stomach contents, the amount of Na_2CO_3 is calculated.

Treatment

Gastric lavage and plenty of fluid containing lemon or grapefruit juice or dilute (1 to 4) vinegar should be given by mouth or with the tube.   Hypodermoclysis with 5 per cent glucose in saline may be given.   Alkalosis should be combated with sodium acid phosphate and stimulants (strychnine and digitalis by hypodermic) for the collapse.

POTASSIUM CHLORATE POISONING

A three year old boy developed a sore throat.   The physician gave directions, over the telephone, to make up a potassium chlorate solution, to be used as a gargle.   The mother misunderstood the directions and gave the child the solution to drink.   (Average fatal dose 3 to 5 grams.)   After several doses the child became seriously ill, with vomiting and diarrhea, clammy skin, dyspnea, cardiac weakness, and a gradual change of the tint of his skin to a pale bluish green, especially pronounced on his lips, nose and forehead.   He died in about six hours.   Icterus and a nephrosis with oliguria have been noted in some cases of chlorate poisoning.

At the postmortem, the skin was found to be grayish blue, the blood chocolate brown in color.   The stomach was not corroded, but of a grayish (pale slate) color.   All the organs were congested and grayish brown in color.   Spectroscopically the blood showed methemoglobin.   The Van Slyke manometric method showed 91.1 per cent methemoglobin.   (Potassium chlorate causes a hemolysis of the blood with the formation of methemoglobin.)   Microscopically the kidneys showed an acute parenchymatous nephrosis.

Detection

The stomach contents, urine, blood, or finely divided tissues are placed into a dialyzing bag made of collodion or parchment paper. Dialysis into pure water is continued for several hours. To some of the dialysate is added an excess of silver nitrate solution. The resulting precipitate of silver chloride is filtered off. The clear filtrate is acidified with nitric acid and sulphurous acid added, and the mixture is then brought to boiling. If a white precipitate remains it is silver chloride from the reduction of the chlorate by the sulfurous acid.

The simplest method for the quantitative estimation of the chlorate is to determine the chloride content of an aliquot portion of the material by one of the usual methods. A second measured portion of the material is made strongly alkaline with added sodium carbonate, well mixed, dried, and carefully ignited at low red heat. This procedure converts the chlorate into chloride. The chloride content is now determined in this ignited material. From the difference between the two chloride determinations, the amount of chlorate originally present can be calculated.

Treatment

Immediate gastric lavage, and administration of copious amounts of fluids is indicated. Nephrosis with suppression of urine and uremia may develop. Stimulating treatment for heart and kidneys should be instituted. Transfusions of blood are of great value.

It is of interest to note that formerly chronic cases of chlorate poisoning resulted from the then common use of chlorate of potash throat lozenges.

ETHYL ALCOHOL POISONING

An infant (aged 4 months) in a hospital received by hypodermoclysis 120 cc. of 95 per cent ethyl alcohol instead of physiological salt solution. The patient immediately began to cry and struggled violently. He became unconscious, the body surface became cold, a subnormal temperature was observed and there were several convulsions, with deep coma, and death after sixty hours. Chemically large amounts of alcohol were found in the organs. It should be noted that children are extremely susceptible to alcohol. Fluids were given by

stomach tube, stimulation with caffein, oxygen, digitalis and strychnine, and heat was applied.   Most of the incidences of alcohol poisoning result from ingestion, in which case gastric lavage must be used.   An interesting case reported by Leschke[6] is that of a four year old child who died from inhalation of ethyl alcohol vapor from an alcohol soaked chest protector.

At postmortem, our patient showed necrosis and sloughing of the skin and subcutaneous tissue at the site of injection.   The heart showed an acute hemorrhagic myocarditis, and hemorrhagic bronchopneumonia was present.   All the other organs were intensely congested.   The brain, after the sixty hour interval, still showed 0.65 per cent (four plus) ethyl alcohol.

Detection

The urine, blood, spinal fluid or finely divided tissue is steam distilled.   About 10 cc. of the distillate is oxidized by repeatedly introducing a red hot copper spiral, cooling the test tube containing the distillate with running water, during this procedure. The oxidation converts the alcohol to acetaldehyde.   When cool, 0.5 cc. of colorless reduced fuchsin is added.   If a red color develops, within 12 to 15 minutes, alcohol was present in the material analyzed.

For the quantitative estimation of alcohol, the steam distillate is oxidized by heating with a sulfuric dichromate mixture, and redistilled.   This procedure converts the alcohol to acetic acid. The latter is then titrated with N/20 sodium hydroxide.   The details of this method are given by Gettler and Tiber.[3,4]

ARSENIC POISONING

Acute arsenic poisoning in children usually results from careless administration, generally of Fowler's solution or accidental ingestion, of the poison.   Cases of chronic arsenic poisoning have resulted from prolonged administration of it, or from prolonged ingestion of foods, for example, arsenic sprayed vegetables and fruits.   The latter cases occur a great deal more frequently than one suspects, and frequently children with vague and obscure symptoms will, especially if the arsenic is mobilized, show arsenic in the urine and feces.

In the acute cases, the symptoms may begin almost immediately after ingestion or only two to three hours later.   This is dependent upon the form of arsenic taken; its concentration, and

whether or not a quantity of food (especially milk) is in the stomach. In the acute cases one generally finds arsenic trioxide, copper arsenite (Paris green), potassium arsenite (Fowler's solution). The symptoms usually commence with severe epigastric pains and vomiting, rapidly followed by burning sensation in mouth and esophagus; profuse bloody diarrhea, so that dehydration and symptoms of collapse (shock) quickly ensue. Convulsions in young children generally occur early, but coma as a rule is a late concomitant. Death is generally thought to be due to paralysis of the muscles of respiration and circulation.

In the chronic cases, usually resulting from prolonged ingestion of minute amounts of arsenic, the symptoms may be very varied indeed, but are chiefly dependent upon disturbances in the nervous system. In this connection, it must not be overlooked that the chronic symptoms may follow months or even years after an acute poisoning. In the chronic cases the most pronounced symptoms are an irritating eczematous eruption, frequently on the extremities, brownish pigmentation of the skin, especially in the groins and the folds of the thighs, and a sensory neuritis, resulting in pains in the extremities and at times, ataxia. Other neurologic disturbances, that may be complained of, are disturbance in vision, sensation of deafness, impairment of taste, severe headaches, sensations of giddiness, spasms and increasing exhaustion, muscular tremors, and loss of the reflexes may be found. Vasomotor disturbances may ensue.

In the acute cases there is generally a brownish discoloration of the upper alimentary tract; frequently petechial hemorrhages and ecchymoses throughout the stomach and intestinal tract, at times so intense that one speaks of hemorrhagic gastritis. General visceral congestion and fatty infiltration (degeneration) in all the organs, but most marked in the kidneys and liver, are generally found. Hemorrhages in the liver and petechial hemorrhages in the pericardium and heart are usually found in the acute cases. Nephrotic lesions are also present in the kidney. Icterus, when the liver has been extensively injured, is present. The brain may show only a few hemorrhages, but as a rule a well-marked hemorrhagic encephalitis is found. In the chronic

cases, although they rarely come to postmortem examination, one generally finds pigmentation and eruptions of the skin, fatty metamorphosis of the liver, and, if a large nerve trunk is examined histologically, a degenerative neuritis may be observed.

The diagnosis can be corroborated during life by finding arsenic in the vomitus, urine and feces, and after death, in the brain, liver, kidneys, stomach and intestinal contents.

Detection

Chemically, the poison is isolated as follows: Urine, feces, stomach contents, or finely divided tissue, are subjected to a nitric sulphuric acid combustion. The completely decomposed, clear, amber colored and nitrate free sulphuric acid solution is diluted with five times its volume of water. This solution is now subjected to the classical Marsh test. A black, lustrous mirror in the cold constricted part of the tube indicates arsenic.

Treatment

In the acute cases treatment must be directed towards elimination of the arsenic by repeated gastric lavage, and administration of the arsenic antidotes (either fresh colloidal ferric hydroxide made by adding an excess of a water suspension of magnesium oxide to a solution of ferric sulphate, or a mixture of equal parts of magnesium oxide and finely divided medicinal charcoal suspended in water) given repeatedly in abundant amounts; also colonic irrigations, hypodermoclysis, saline and glucose intravenously, and transfusions to combat the dehydration from the diarrhea which usually develops. Supportive drugs must be employed if there are symptoms of collapse or cardiac or respiratory failure. In the chronic cases efforts must be employed to determine the source of the poison, and then avoid any further contact of poison with the patient. At the same time continued attention should be directed toward the elimination of the arsenic from the system by giving the patient an acid residue diet, in which milk is excluded, and also intravenous injections of sodium thiosulphate. If the neuritis is very marked, physio- and electro-therapy should be employed. In this connection, attention

must be called to the fact that these patients have as their predominant symptoms, lack of physical and mental energy, easy fatigue, lassitude, and a general vagueness of feeling unwell. This must be combated by detailed attention to the general hygiene and by giving stimulating tonics and vitamine-rich diets.

It has been estimated, especially in children, that over three-quarters of the cases of chronic arsenical poisoning go unrecognized, and the physician, believing that these patients require a tonic, prescribes either orally or by injection one of the many preparations of arsenic that are found in every pharmacy. Thus, when arsenic is found in one or other of the excreta, a careful inquiry should be made as to what medication has recently been employed.

PHOSPHORUS POISONING

Among our cases we have had one of acute phosphorus poisoning in a two and one-half year old child who had eaten a quantity of home-made sweetened roach paste containing phophorus as a principal ingredient. It was estimated that thirty-six hours after ingestion, the child suddenly developed vomiting, repeated convulsions, diarrhea, jaundice, coma, collapse, and death within fifty hours. Treatment was of no avail. At necropsy, a soft butter-yellow, small liver, with diffuse hemorrhagic areas, was the principal lesion. Congestion of all the viscera and petechial hemorrhages in the serous membranes are also generally found. It is of interest to note that the vomitus and eructations have a garlic like odor and the vomitus may show a phosphorescence in a dark room. Chronic phosphorus poisoning, the so-called phosphorus jaw, has never been described in children.

Detection

In acute (white) phosphorus poisoning, the gastro-intestinal contents is of prime importance from an analytical standpoint. The material is distilled in a dark room screening off the light from the Bunsen burner. If white phosphorus is present, a luminescent ring will be noticed during the distillation, in the upper part of the water cooled condensor. For a further test, the distillate is strongly acidified with concentrated nitric acid and then evaporated to dryness on the water bath. This procedure converts the phosphorus to phosphoric acid, which can then be identified by the ammonium molybdate reaction.

Treatment

Generally little is accomplished, since the symptoms do not occur until a good deal of the poison has been absorbed and has exerted its deleterious action.   Acute phosphorus poisoning may be divided into three stages: (1) Acute gastro-intestinal symptoms; (2) Quiescent stage lasting a day or two; (3) Symptoms similar to an acute yellow atrophy of the liver.   Treatment is directed towards elimination, support and stimulation.   Morphine or barbiturates should be given liberally in case of great pain.   Large amounts of liquid petrolatum should be administered, and then removed by siphonage with the stomach tube. Repeated lavage with a 1 per cent solution of potassium permanganate should next be instituted.   Suspension of finely divided medicinal charcoal, in large amounts, should finally be given.

ANILINE AND NITROBENZENE POISONING

A most interesting case of aniline and benzene poisoning at Bellevue Hospital was reported by Graves.[5]   A girl aged five with a negative history was admitted to the hospital in partial collapse about 4 p.m.   The patient seemed well until 2 p.m.   Then developed headache while riding in subway accompanied by her mother.   The patient and mother had been to a restaurant and had an ordinary dinner.   As they were leaving the restaurant the mother noticed that the child had lost her normal color, and a few minutes later the child collapsed in the street.   An ambulance brought her to the hospital.   At the hospital the examination revealed a well nourished child, in semi-stupor, with circulatory weakness. Temperature, 98°; pulse, 104; respirations, 20.   The quality of pulse was not good, heart irregular, lungs not obstructed; there was a general cyanosis, most marked in the lips, tongue and fingertips.   There was a leucocytosis of 20,000, a hemoglobin content of 82 per cent, and a trace of albumin in the urine.   At 8 p.m., the patient was still in a semi-stupor, and cyanotic with a weak irregular pulse.   Treatment given was digifolin, caffein, sodium benzoate, atropine, and a mustard bath.   At this juncture it was suggested that an extrinsic toxic agent was responsible.   The mother denied contact with gas, headache powders, paint, radiator enamel, roach paste, roach powder, et cetera.   Finally, after much questioning, the mother remembered that the child had donned a newly-dyed pair of suede shoes.   Thereupon 10 cc. of blood was removed from the patient.   The blood had a mahogany color and showed methemoglobin with the spectroscope.   At 11 p.m. a transfusion was given.   There was almost immediate transformation for the better.   The child left the hospital on the second day entirely recovered.   Analysis of the shoe dye revealed aniline, and benzene.

Aniline in the body forms phenylhydroxyamine, which substance then produces methemoglobin, after the hemolysis of the erythrocytes.

The principal symptoms are fatigue, nausea, headache, giddiness, tinnitus aureum, skin irritation and sleeplessness. There may be paralysis, abdominal cramps, absent reflexes, unconsciousness, palpitation and dyspnoea. The prominent symptoms are an anemic pallor and cyanosis; the skin is sometimes deep blue, and at times it and the eyes are icteric due to bilirubin liberation. The peculiar discoloration of the skin is almost diagnostic. The urine contains methemoglobin, hemoglobin, porphyrin, bilirubin, albumin and casts. There is nothing especially characteristic in the pathology. Anemia, parenchymatous degeneration of the organs, hemorrhage in serous membranes, blood changes (methemoglobin), pinhead cerebral hemorrhages may be found. The liver may resemble an acute yellow atrophy with small patches of necrosis.

Detection

For the detection of aniline and nitrobenzene, the finely divided organs, urine, blood, or stomach contents are distilled with steam. If these substances are present in appreciable amount, the distillate is usually cloudy. To one portion of the distillate add two drops of chloroform and excess NaOH solution, and bring to boiling. If aniline is present, the very irritating and piercing odor of phenyl isocyanide develops. Should nitrobenzene be present, the steam distillate is shaken out with ether. The ether layer is allowed to evaporate spontaneously. If nitrobenzene was present, it will now be found in the form of a few oily globules with the characteristic odor of oil of bitter almonds. These drops are dissolved in alcohol. The alcoholic solution is reduced with powdered zinc and HCl. This converts the nitrobenzene to aniline. After making the solution alkaline with NaOH, the aniline obtained is tested by means of the isonitrile reaction as above.

Treatment

Remove the source of the poison at once.   If on the skin, wash off with soap and water; if taken by mouth, wash out the stomach and give suspension of medicinal charcoal in water and magnesium sulphate in liberal amounts.   Camphor and caffeine preparations, oxygen, saline infusion, and in an emergency, artificial respiration should be used.   Transfusions are of tremendous value in this type of poison, and should be given promptly.   For the subsequent anemia, liver extracts intramuscularly and by mouth should be used.

BENZENE POISONING

A boy, aged 13, was in the habit of frequenting a shoemaker's establishment. To the rear of the store was a small room in which the proprietor kept his rubber cement.   The boy spent much of his time in this room.   One cold winter's day, he remained in the room much longer than usual.   The proprietor opened the door to see what was going on, and found him collapsed over the table.   On examining the scene, the medical examiner found a large 5 gallon tin can with the cap off, standing on the table.   A large stain was found on the table and floor.   The necropsy and chemical analysis proved death due to benzene poisoning.   The boy had evidently upset the tin can containing the rubber cement with benzene the solvent.   The benzene evaporated into the air of the room.   The door and windows were closed.   The boy kept inhaling the vapors, became dizzy, unconscious and died.

Benzene poisoning may be of two varieties: acute and chronic. The acute type, resulting from the ingestion of a large amount of benzene at one time, commences with a state of inebriation in which gaiety, excitement and increased self-confidence precede a state of giddiness, uncertain gait, sleepiness, nausea, vomiting, headache, facial pallor, later assuming a grayish-blue tint, with cyanosis of the extremities, muscular spasms, slowing of respiration, fall in temperature and blood pressure, finally, muscular paralyses, convulsions, deep coma and death.   Burning throat and eyes are frequently complained of.

In the chronic type of poisoning, usually occurring from prolonged inhalation of the poison or ingestion of small amounts in liquids (denatured alcohol), et cetera, fine punctate hemorrhages, giving the appearance of purpura hemorrhagica are extensively

scattered all over the skin, and mucous membranes of nose, mouth, et cetera. In addition, there is a lack of appetite, nausea with the desire to vomit, and headaches are quite frequent accompaniments. Neurological symptoms, especially tremors, faulty gait, fatigue, giddiness, abdominal cramps and pains, insomnia and cardiac palpitation are often encountered. The urine generally shows small amounts of albumin and granular casts, and in some cases a retro-bulbar or polyneuritis or even median nerve paralysis results. In the cases ending fatally, a generalized sepsis, due to infection of the hemorrhagic foci, is frequently found. A characteristic blood finding may be at first a slight or moderate leukocytosis, which is quickly followed by a decided leukopenia with a relative lymphocytosis (similar to agranulocytic angina of Schultz) and anemia. The leukopenia is characteristic of benzene poisoning and caused by an aplasia of the bone marrow.

At necropsy in the acute type of poisoning, the skin frequently shows bright red "spots" with a marked chronic passive congestion. Pial edema and pulmonary edema are frequently quite pronounced. In addition, hemorrhages into such tissues as the skin, serous and mucous membranes, into the meninges and even into the brain tissue, nerves and retina are quite pronounced.

In the chronically poisoned body the most typical finding is the reddish-yellow gelatinous appearing aplastic bone marrow. In addition degeneration and necrosis of the spleen and lymph nodes is found together with hemorrhagic foci in the skin, mucous membranes, heart muscle, liver and adrenal glands.

Of interest are the blood and bone narrow studies. Depending on the severity of the intoxication one may find anisocytosis, poikilocytosis, polychromatophilia, and basophilic stippling. Reticulocytes are always absent and marked platelet destruction is found. Generally, there is considerable pigment deposition in both the spleen and liver and actual destruction of erythrocytes can be observed. As a result of these changes both the bleeding time and coagulation time are increased in these individuals, and they exhibit a tendency to bleeding. The development of the changes in the erythrocytes together with the leukopenia and

relative lymphocytosis are always signs of serious import, and in the majority of the cases lead to a fatal termination. As a result of these changes, pigmentation, eczema and other chronic irritative inflammations (cornification and keratosis) of the skin and its follicles have been found.

Detection

For the detection of benzene in tissues, the simplest procedure is that recommended by Gettler and Siegel[2].

Treatment

In both types of cases the sources of benzene must be eliminated. In the acute cases, if the patient is unconscious, artificial respiration or the use of the respirator with oxygen and carbondioxide administration should be employed. Stimulants for the heart and respiration should also be given. If the patient has taken the benzene by mouth, a suspension (3 per cent) of medicinal charcoal in water is used as a gastric lavage. This should be followed by the liberal administration of a suspension of medicinal charcoal in magnesium sulphate solution.

If the poisoning has resulted from inhalation, attempts have been made to bind the benzene by the intravenous injection of 5 cc. of a 10 per cent lecithin emulsion. Its use, however, is of doubtful value.

In the chronic cases, measures directed towards combating the anemia and overcoming the bone marrow injury must be employed. This is accomplished chiefly with transfusions, daily injections of liver extract, administration of hematinics and proper diets.

STRYCHNINE POISONING

A child, aged 15 years, who, while in the hospital was given through an error of the hospital pharmacist, iron-strychnine citrate, instead of iron ammonium citrate. Within a half hour the patient developed typical symptoms of strychnine poisoning; he became restless and agitated, then complained of muscular symptoms. This was quickly followed by muscular twitchings, opisthotonus, cyanosis, intermittent respirations, convulsions and death. Sedatives and inhalations of chloroform were given, but were of no avail. The resemblances to tetany may lead to an error in diagnosis in such cases.

Detection

The finely divided tissues, stomach contents, or urine (which has been evaporated to a syrup), is acidified with hydrochloric acid and treated with five times the volume of ethyl alcohol, and allowed to stand several hours with occasional shaking. The material is then filtered. The filtrate is evaporated to a thick syrup on the water bath. To the residue, about 200 cc. of alcohol are added gradually with constant stirring, and then allowed to stand about one hour. It is then filtered, and the filtrate evaporated on a water bath until it no longer smells of alcohol. The residue is taken up with about 100 cc. of warm water, stirring well. It is then filtered to remove all insoluble material. The filtrate is shaken out three times, in a separatory funnel, with 50 cc. portions of ether; the ether layers each time are discarded. The aqueous layer is now made alkaline with NaOH solution, and shaken out three times with fresh 50 cc. portions of ether. The three ether extracts are combined and washed in a separatory funnel with a small volume of water. The ether layer is then filtered through a dry filter paper, and evaporated to about 5 cc. on a water bath. A few drops of this concentrated ether extract are placed on a small watch glass and allowed to evaporate. If strychnine was present it will now be found as a white crystalline powder on the watch glass. If the amount of strychnine is very small, the residue may not have the above appearance. To the residue on the watch glass, apply a drop of Mandelin's reagent (ammonium vanadate dissolved in concentrated sulphuric acid). A play of color results, deep blue, royal purple, violet and lastly after about 5 minutes, an orange color. The final orange color is essential, in order to rule out pseudo-reactions.

Treatment

Recently Stalberg and Davidson successfully treated a severe case of strychnine poisoning by injecting sodium amytal intravenously and tribrom-ethanol in water was given by rectum. Gastric lavage with a suspension of medicinal charcoal and tannic acid should be given at once. Chloral hydrate, as well as ether

or chloroform may be given for the spasms. An intravenous injection of pernokton may be given in drop doses.

LEAD POISONING

We have recently had an interesting case of a 2½ year old male child admitted to a hospital in a neighboring city with a history of continued convulsions of only a few hours' duration. Examination of a blood smear made in the course of a routine blood count revealed intense basophilic stippling. This made the interne suspicious of lead poisoning, and careful inquiry from the mother elicited the fact that the child had bitten and scratched a good deal of the paint off his enameled bed. We examined the urine and feces for lead and found considerable quantities of it.

Necropsy, about 48 hours after admission, revealed marked encephalitis and considerable quantities of lead in a small piece of the femur.

The type of lead poisoning in children is usually more or less of the chronic variety and generally occurs by way of the gastro-intestinal tract, rarely through the skin and practically never by means of inhalation. The source of the lead is from the lead paint on furniture, wood-work or toys.

The cases of acute lead poisoning show symptoms of acute gastro-intestinal disturbances, such as colic, abdominal cramps, and pains, nausea, vomiting and usually constipation. (At times a differential diagnosis from acute appendicitis is difficult.) These symptoms are usually rapidly followed with signs of cerebral disturbance, convulsions, muscular twitchings and coma. An acute hemolytic anemia has been observed in some cases.

The repeated ingestion of small amounts of lead produces signs of absorption such as the blue line on the gums, and changes in the radiological appearances of the long bones, without producing any signs of intoxication. This state of latent poisoning is found more often among adults than in children, the young being relatively more susceptible to lead. The amount of lead absorbed, the pH of the blood and idiosyncrasy are the factors influencing intoxication.

The disturbance of the health produced by chronic lead poisoning causes such general symptoms as constipation, vomiting, loss of appetite, and irritability, which is often extreme. These symptoms usually insidious in onset, are found at varying periods

after absorption has begun. A secondary anemia is a constant accompaniment, but basophilic stippling, though usually well marked, is not an invariable finding. The blue line, though important diagnostically, is not often seen in children.

In these phases of intoxication, an infection or other factor giving rise to acidosis, frequently precipitates symptoms of an acute attack, particularly encephalitis.

Unlike the adult, children manifest paralyses most often in the lower rather than in the upper limbs, presumably because in children the legs are more often fatigued than the arms. Pains and reflex changes may accompany the weakness. Papilledema and ocular palsies are usually found in encephalopathy, though they may occur alone. Optic atrophy sometimes results in untreated cases, but the ocular palsies always recover.

Lead encephalophathy is the most serious of the acute manifestations of poisoning in children. The symptoms caused by the acute cerebral edema are those of a raised intracranial pressure with degression and excitation of the brain. There is headache, vomiting, papilledema, and a bulging fontanelle with stupor or coma. There may be paralyses of the ocular or limb muscles and the children are sometimes delirious or tremulous. Convulsions and twitching are common and have been noted to occur in groups. In many cases there are, in addition, signs of meningeal irritation such as rigidity of the neck and retraction of the head, and this may be reflected in the cerebro-spinal fluid by a pleocytosis. The fluid is always under increased pressure and the protein content is raised, but the rise in cell content is not constant. Fever, which is often present, is due in most cases to an accompanying infection.

The prognosis in encephalopathy is serious. Mental deficiency, convulsions, blindness from optic atrophy, and cerebral palsies have all resulted from the ischemia produced during lead encephalopathy by the acute cerebral edema. It is believed that chronic interstitial nephritis in children may be due to lead poisoning earlier in life. Another late effect to be noted is renal dwarfism.

Radiographic changes produced by lead in the long bones of

children appear as a band of increased density at the diaphyseal end of the growing bone, most marked where the growth is greatest. The outline of the bone is unchanged and there is no evidence of irregularity at the epiphyseal junction or of raising of the periosteum. The epiphyses are quite normal. The degree of opacity and the width of the dense band varies with the amount of lead absorbed and the length of time tends to become wider and less well-defined. The density is due to the deposition of lead in the bone chiefly and also to the condensation of the trabeculae. If the absorption of lead ceases, an area of bone of normal density appears on the epiphyseal side of the "lead line." As the metal is deposited only in the growing bone the changes are not seen in adults.

Similar, but not identical, appearances are seen in scurvy, congenital syphilis, Albers-Schonberg's diseases (marble bones), and in poisoning by vitamin D and elementary phosphorus, and a few cases have been reported where other heavy metals, such as strontium and bismuth have been the cause. The clinical features in these conditions are usually sufficiently distinct to avoid confusion.

At necropsy these children generally show an encephalitis and an intense congestion of the viscera and at times spastically contracted intestines.

Three types of cases in which lead poisoning may be suspected are : (1) convulsions of obscure origin, or cases of sterile meningitis; (2) cases showing papilledema with or without ocular palsy, for which no cause can be found, and (3) anemic children who suffer from colic, constipation and irritability or in whom signs of peripheral limb palsy are found. In the diagnosis of a suspected case of poisoning a history of the child having ingested lead in some form is important and usually can be obtained.

Aub, Fairhall, Minot and Reznikoff[1] and Rogers, Peck and Jupe[7] in their papers on lead poisoning in children present an excellent review of the subject.

Detection

The urine, feces, stomach contents, or finely divided organs are dried on the water bath and then ashed in a muffle furnace

at low red heat. The ash is extracted by boiling with hydrochloric acid solution (1 to 1) for about fifteen minutes. An equal volume of water is then added, brought to a boil and filtered hot. The filtrate is neutralized with sodium hydroxide solution. A precipitate due to phosphate usually forms, hydrochloric acid is added dropwise until this just clears up. Hydrogen sulphide is now passed into the solution for 10 minutes. It is stoppered and allowed to stand overnight. The next day the sulfides are filtered off and washed, then dissolved in a small amount of hot (1 to 1) nitric acid. To the nitric acid solution in a small beaker are added about 3 cc. concentrated sulphuric acid. The solution is then evaporated by boiling until fumes of SO_3 evolve. Allow to cool; add equal volume of water and heat until SO_3 fumes arise again, in order to drive off the last traces of nitric acid. When cool, add about 40 cc. of water and allow to stand overnight. The next day, a small crystalline sediment may be found on the bottom of the beaker. This indicates the presence of $PbSO_4$. To further prove, carefully siphon off or pour off most of the supernatant liquid without disturbing the precipitate. The sediment is now transferred to a centrifuge tube, and centrifuged for 10 minutes. The supernatant liquid is discarded. To the sediment in tube 1 cc. of a saturated solution of ammonium acetate is added, and the tube placed in a boiling water bath for 5 minutes, stirring occasionally. The tube is now removed, an equal volume of water added, mixed and filtered. To the filtrate, one or two drops of potassium chromate solution are added. If lead is present a canary yellow precipitate results. If all of the above procedures were carried out quantitatively, the $PbCrO_4$ can be filtered through a micro filtering stick, washed and weighed, for a quantitative determination. For volumetric and micro procedures one should refer to the publication by Aub, Fairhall, Minot and Reznikoff.[1]

Treatment

In the acute type of lead poisoning, the lead should be removed from the gastro-intestinal tract by repeated washing with 1 per cent sulphuric acid or 5 per cent sodium sulphate solution. The circulating lead should be removed as quickly as possible,

since this cannot be done by excretion, it is best accomplished by depositing the lead in organs and bones. For this purpose an alkaline diet must be given (large amounts of milk, fruits, vegetables and potatoes), also calcium gluconate in milk every three hours (0.5 to 1.0 gram calcium gluconate). After a period of rest, mobilization can be commenced by administering an acid residue diet (large amounts of meat, coffee, tea and cereals—no milk or calcium). Twenty-five cubic centimeters of N/10 phosphoric acid in a glass of raspberry syrup may be given with this. Laxatives (magnesium sulphate) should also be given.

Administration of *ammonium chloride* for the liberation of the lead has lately been shown by Litzner, Weyrauch and Barth, to be of little value. Potassium iodide seems to be best for this purpose. The latest observations seem to point to the use of smaller doses, so that the lead shall not be liberated into the circulation in too great an amount at any one time. Sodium iodide 0.5 grams dissolved in milk given with meals three times a day, and increased to four or five times this dose if tolerated, should be given. Intramuscularly, iodisan, or mirion may be given and intravenously septoiodin (iodide, iodate, hypoiodite). Sodium bicarbonate makes lead phosphate more soluble, and 20 to 30 grams are given in five doses. This gives very rapid mobilization, hence, it is better to use the iodide first, and the bicarbonate later in order to get out the last traces of lead. The mobilization treatment should be administered intermittently and stopped the moment symptoms of lead intoxication become aggravated. Thiosulphate preparations given intravenously are only effective in acute or recent incidences of poisoning. Recently colloidal sulphur has been employed but further investigation is required before it can be used. This also applies to the parathormone therapy.

Lead colics are best treated by intravenous injections of 5 to 8 cc. of 10 per cent calcium bromide. If injected slowly the feeling of heat is less intense than if one uses calcium chloride, hence one can dispense with the injection of opium or scopolamine. Novatropine and eupaverine are given internally after meals as antispasmodics. Purgatives to be used are senna, sodium sulphate, petrolagar; these cause no spasms.

In children the treatment of lead encephalopathy is important. All sources of lead should be removed and the child be guarded carefully against further risks of poisoning. Intracranial pressure must be lowered to safe limits by lumbar puncture. The injection of hypertonic solutions have proved disappointing. A 15 per cent saline solution or a 25 per cent solution of glucose in normal saline may be given intravenously in doses of about 50 cc. to a child of five years. The solution may be administered rectally and is of some value if retained for more than 30 minutes. In cases of delirium or convulsions, sedative should be given in addition to the other treatment.

SUMMARY

We have by no means exhausted all the poisons and poisonings which are found in childhood, and the physician, pathologist and toxicologist must realize that the accident of poisoning in childhood may be brought about by any and every poison, dependent upon a great many causes and factors. For example, we have not touched upon the poisoning of the suckling infant who may receive poison through the mother's milk.

We believe we have, however, enumerated the greatest number of common causes of poisoning in childhood and have sufficiently illustrated these with case histories and personal experiences, so that the physician, when called upon to attend such a patient, will have a fairly good conception of what he should do and what he may hope to accomplish. We have also included simple analytical procedures for the detection of the poison.

In conclusion we wish to call attention to the importance of saving vomitus, and the first gastric lavages, urine and feces, and having these chemically examined during life in suspected cases of poisoning. One should also save gastro-intestinal contents and large portions of the brain, liver and kidneys for toxicological analysis, when these are obtained at necropsy.

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THE CLINICAL PATHOLOGY OF RHEUMATOID ARTHRITIS*

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Rheumatoid arthritis is the most common and the most devastating of the chronic arthritides. This is particularly true in the North temperate climate and along the North Atlantic seaboard, where there are sudden changes in temperature and wide variation in barometric pressure and where upper respiratory infection is common.

The rheumatoid type of arthritis, otherwise known as chronic infectious arthritis, atrophic arthritis, proliferative arthritis and arthritis deformans, should be distinguished from osteoarthritis, otherwise known as non-infectious, hypertrophic, degenerative and senescent arthritis.[4]

In this climate it will be found that the first symptoms of rheumatoid arthritis most frequently, and often dramatically, follow acute attacks of "head colds" and sore throat. It is obvious that such cases seen for the first time five years after the onset are not suitable for etiologic studies because at that time the primary focus may be quiescent or absent, and complicating conditions such as muscle atrophy and intestinal stasis may lead to false conclusions.

We know from the study of tissue removed at operation and at necropsy that the first change in these joints occurs in the synovial and periarticular tissue and that this change is characterized by the infiltration of leucocytes and the proliferation of fibroblasts. This productive inflammatory reaction is shown in the photomicrographs (figs. 1 and 2), prepared from sections of thickened synovial tissue of one of our cases that came to necropsy.

For a long time many observers believed that the periarticular inflammation in rheumatoid arthritis was of an infectious nature but they failed to show the connection between the pathological process in the joints and the systemic or focal infection.   Thirteen years ago Rosenow[8] isolated a streptococcus from enlarged lymph nodes adjacent to diseased joints.   Forkner, Shands and Poster[3]

FIG. 1. PHOTOMICROGRAPH PREPARED FROM A SECTION OF THE SYNOVIAL TISSUE

It shows necrosis near joint cavity, cellular infiltration, increased vascularity and fibrous production in a case of rheumatoid arthritis.   ($\times 186$.)

in 1928, found streptococci in joint cultures in 16 per cent of 63 cases.   Prior to the cultural studies of blood and joint fluid by Cecil, Nichols and Stainsby,[2] we tried various culture methods with but little success.   After that time we not only succeeded in duplicating their results by isolating streptococci from the blood and joint fluid in a high percentage of cases, but also modified the method in such a manner that a growth could be

obtained in a short period of time without the necessity of opening the bottles or subculturing.

Our method has been described in detail elsewhere,[5,6,7] but the preparation of the medium may be briefly summarized as follows:

    (1) Grind finely 100 grams fresh beef heart.

    (2) Add 1000 cc. of water.

    (3) Infuse at 15 to 20°C. overnight.

    (4) Warm to 20 to 25°C. and squeeze through flannel bag.

    (5) Boil filtrate slowly 1 hour and filter through filter paper.

    (6) Make up to volume.

    (7) Add 1.5 per cent peptone, 0.5 per cent NaCl, 1 per cent dextrose and 1 per cent gelatine.

    (8) Dissolve in Arnold sterilizer 20 to 25 minutes.

    (9) Adjust to pH 8, Arnold sterilize 1 hour, filter through filter paper.

    (10) If pH is below 7.8, adjust to that figure.

    (11) Prepare sterile bottles adding 10 to 15 grams of marble to each.

    (12) Place 50 to 60 cc. in each bottle (depth media, 30 mm.).

    (13) Arnold sterilize 30 minutes on three successive days.

    (14) If pH is 7.6 to 7.8 it is satisfactory for use.

    (15) Incubate several days for sterility.

The organisms recovered from the blood in rheumatoid arthritis are for the most part alpha or alpha prime streptococci, most of which do not thrive in free oxygen, and for that reason our culture technic requires large amounts of broth in deep bottles and the addition of gelatine for creating oxygen tension. Growth may also be enhanced by discarding the serum which contains native complement and using only the clot in the culture preparation. Furthermore, a well buffered medium is desired for the purpose of absorbing the large amount of acid which these organisms produce. Great care must be taken in selecting fresh beef hearts and no pressure should be used in sterilizing the finished product.

The blood culture is carried out in the following manner:

Twenty cubic centimeters of blood, drawn from the patient's vein, is allowed to clot overnight in the refrigerator. The next morning the tubes are shaken to loosen the clots, centrifuged, the serum pipetted off and the clot broken up and transferred to a culture bottle which is thoroughly shaken and incubated. The

cultures are inspected every 24 hours for diffuse cloudiness and dark brown discoloration which appears in from 1 to 6 days in positive cultures.

For the identification and further study of the organisms blood agar plates are used. They are prepared from a sugar-free fresh heart infusion medium containing 1.5 per cent agar with a pH of 7.4. This is stored in amounts of 100 cc. and when desired for use the medium is melted, cooled to 45°C. and 5 cc. of citrated human, rabbit or sheep blood is added. About 10 cc. is poured into each plate and they are cooled in the refrigerator before streaking.
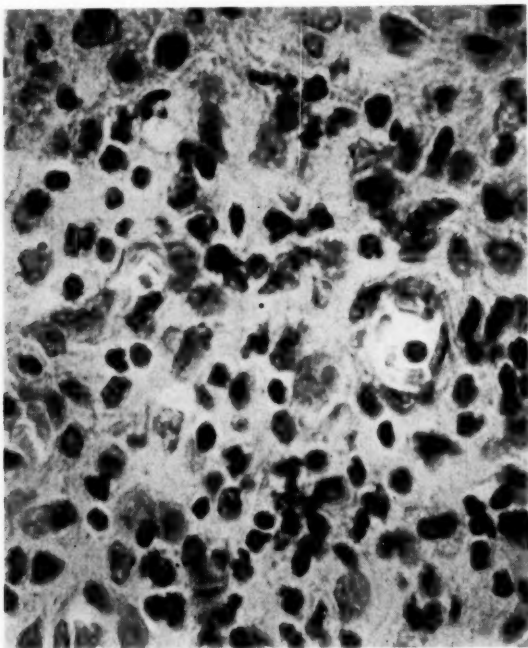
FIG. 2. HIGH POWER FIELD OF A CELLULAR AREA OF FIGURE 1

It shows lymphocytes, plasma cell infiltration and fibroblastic proliferation

Stained specimens from the original cloudy bottle shows the formation of long chains during the first 24 hours of growth (fig. 3), which after that length of time tend to become broken up into short chains (fig. 4). A good growth usually develops on streaked blood agar plates in from 24 to 48 hours but a blood broth tube is also inoculated since an occasional strain will not develop satisfactorily on plates until it has been planted in blood broth for several generations.

Alpha prime colonies (fig. 5) are grayish in color and oval.   There is a small amount of methemoglobin found and, after 24 to 48 hours incubation, a light ring of incomplete hemolysis appears.   For the detection of this slight hemolysis a hand lens may be needed but after removing the colony a definite area of hemolysis is easily visible and the hemolytic tendency becomes more and more pronounced on succesive transplants.

The alpha colonies (fig. 6) are raised and pointed, grayish green in color and show a definite production of methemoglobin but no zone of hemolysis.   Even after repeated transplants hemolysis does not appear.

FIG. 3   FIG. 4

FIG. 3. PHOTOMICROGRAPH OF ALPHA PRIME STREPTOCOCCUS FROM 18-HOUR CARBOHYDRATE BROTH CULTURE

FIG. 4. PHOTOMICROGRAPH OF ALPHA PRIME STREPTOCOCCUS FROM 48-HOUR CARBOHYDRATE BROTH CULTURE

It may be seen from table 1 that in a consecutive series of 200 rheumatoid arthritis cases streptococci were cultured from the blood in 48 per cent, and in 65 per cent of the ninety-two early cases.   A small percentage of staphylococci and diphtheroids also appeared in these cultures, the significance of which cannot be determined without further investigation.   There were also seventy-nine cases of osteoarthritis cultured during the same period of time and all were negative for streptococcus.

Control cultures indicated in table 2 consisted of thirty-six normal individuals in which no growth was found, twenty-two cases of acute focal infection with 23 per cent positive results, twenty-six chronic focal infection cases with 4 per cent positive,
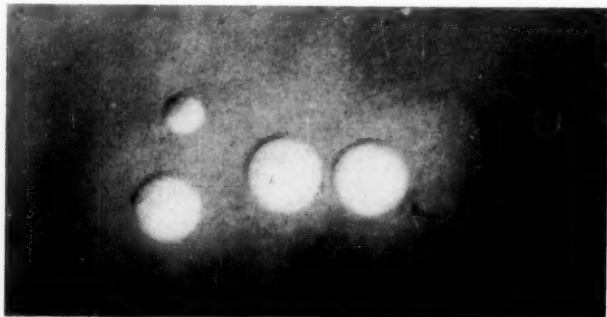
FIG. 5. PHOTOMICROGRAPH OF ALPHA PRIME STREPTOCOCCUS COLONIES

Blood agar plate showing flat colonies and a faint halo of hemolysis
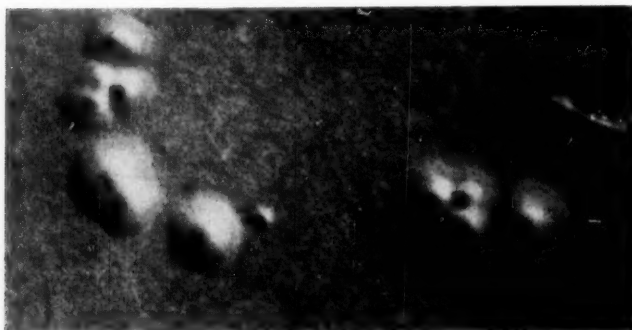
FIG. 6. PHOTOMICROGRAPH OF ALPHA STREPTOCOCCUS COLONIES ON A BLOOD AGAR PLATE

The colonies are pointed and show no hemolysis

and eighty-nine cases of so-called arthralgia in which 8 per cent showed a growth of streptococci. Cases of acute focal infection included seven of acute follicular tonsillitis, two of which were positive, and fifteen of acute sinusitis, three of which were

positive.   In the group of chronic focal infection there were
twelve cases of chronic sinusitis, nine chronic tonsillitis, two
chronic cholecystitis, two chronic ulcerative colitis and one
chronic pyelitis.

The large group of eighty-nine cases of arthralgia in this control
group, distinguished from arthritis by the absence of swelling and
other evidence of joint pathology, showed a streptococcal growth

TABLE 1

BLOOD CULTURES FOR STREPTOCOCCI IN CHRONIC ARTHRITIS

DIAGNOSIS	CASES	CULTURES	CULTURES		
			Negative	Positive	Positive
					per cent
Rheumatoid arthritis.............	200	308	213	95	48
Early.........................	92	137	77	60	65
Established...................	63	106	83	23	36
Advanced.....................	45	65	53	12	26
Osteoarthritis..................	79	95	95	0	0

TABLE 2

CONTROL BLOOD CULTURES FOR STREPTOCOCCI

DIAGNOSIS	CASES	CULTURES	CULTURES		
			Negative	Positive	Positive
					per cent
Normal individuals..............	36	48	48	0	0
Acute focal infection.............	22	26	21	5	23
Chronic focal infection...........	26	33	32	1	4
Arthralgia.....................	89	108	101	7	8

in 8 per cent.   Focal infection was almost as prevalent in this
group as in the rheumatoid group, showing that although bacte-
remia may be associated with focal infection and arthralgia, it is
much more common when the joint infection becomes established.
This observation was more or less corroborated by negative cul-
tures from the joint fluid in early cases and positive cultures in
more advanced cases.

It is of considerable interest that relatively few positive cultures

were obtained in rheumatoid cases during the summer months. In many cases cultures from tonsils, teeth and sinuses yield streptococci of the same group as found in blood and joint fluid cultures.

Further evidence of streptococcal infection in rheumatoid arthritis was found in agglutination tests illustrated in table 3.

In most cases the agglutination was rather marked and the clumps large and not easily broken up.   In some cases, there was a pro-agglutinoid zone extending as high as 1:80 and a few showed agglutination beyond the 1:5120 dilution.

This procedure is apparently very reliable in detecting antibodies to more or less specific strains of streptococci and may be

TABLE 3

AGGLUTINATION TESTS WITH STREPTOCOCCI

DIAGNOSIS	DILUTIONS OF SERUM		
	0–80	160–320	640–5120
	per cent	per cent	per cent
Rheumatoid arthritis:			
Early	24	17	59
Established	26	20	54
Advanced	30	17	53
Osteoarthritis	83	17	0
Arthralgia	48	36	16

compared to the Widal reaction in typhoid fever.   In our rheumatoid cases 70 to 76 per cent showed readings from 160 to 5120, and in osteoarthritis there were no high titre readings in uncomplicated cases.   The interpretation, however, from a clinical point of view is quite difficult.   Why the titre increases in some cases that are progressing satisfactorily while it decreases in others, and why it may increase one month and decrease the next month, is not known.

The sedimentation rate is one of the most valuable tests for the diagnosis of rheumatoid arthritis.   It indicates activity of a disease process, usually associated with infection and is of value not only in the differential diagnosis of rheumatoid arthritis and osteoarthritis, but is also helpful in studying the effects of treat-

ment in the former disease.   Rheumatoid arthritis always shows an accelerated rate if the disease is active and as progress is made in controlling the infection the rate decreases until it approaches normal.   We employ the Westergren method of determining the sedimentation rate because of its wide reading scale and each specimen is corrected to a constant cell volume and constant temperature.

In table 4 the high sedimentation rates in rheumatoid arthritis and low rates in osteoarthritis are significantly shown.

Active serum complement fixation was first applied to cases of arthritis by Burbank and Hadjopoulos[1] and may be useful in detecting the type of streptococcus or other organisms where the

TABLE 4

CORRECTED SEDIMENTATION RATES

DIAGNOSIS	MILLIMETERS PER HOUR		
	0-10	10-20	20+
	per cent	per cent	per cent
Rheumatoid arthritis:			
Early.................................	23	37	40
Established...........................	14	25	61
Advanced.............................	8	25	67
Osteoarthritis.........................	85	13	2

agglutination reaction is negative or very low.   Theoretically, the thermo-labile antibodies are not destroyed by inactivation and these combine with the homologous bacterial antigen, thus fixing the native human complement and causing inhibition of hemolysis and a positive result.   Practically it is subject to considerable error unless rigidly controlled.   This procedure was applied in a small group of our cases and the results are given in table 5.

Electrophoresis studies, as yet too complicated for general use, are also of value in detecting reactions between antibodies and homologous bacteria and are destined to play an important part in the bacteriology and vaccine treatment of rheumatoid arthritis.

In a few of our cases of rheumatoid arthritis the administration

of even the most minute dose of specific vaccine was not well tolerated.   In these cases the complement was usually found to be lower than normal and unless the titre could be increased by appropriate measures, such as transfusions, rest, heliotherapy, high vitamin and nutritious diet, further administration of specific vaccine apparently produced a "negative phase" and was

TABLE 5
Active Serum Complement Fixation

CASE	ANTIGENS						
	Streptococci				Staphylo-coccus	Colon bacillus	Gono-coccus
	Beta	Alpha prime	Alpha	Gamma			
Rheumatoid							
1	+++	++	+++	+++	++	++	0
2	++++	+++	++++	++++	++	+++	0
3	0	++	+++	+	0	+	0
4	+	±	0	0	+	0	0
5	++++	++	++++	++	++++	++++	±
Osteoarthritis							
6	0	0	0	0	0	0	0
7	+	0	0	0	0	0	0
Arthralgia							
8	0	0	0	0	+	0	0
9	+++	+	++	+++	++	+++	0
Focal infection							
10	0	0	0	0	0	0	0
11	0	+	0	0	0	0	0
12	++	++	++	++	+++	+	++++
13	0	0	0	0	0	0	0

harmful to the patient.   Table 6 shows the variations in the sera of fourteen patients.

One of the most important objects of cultures, serological tests and cataphoresis studies is the selection of a suitable organism for vaccine preparation.   An organism isolated from the patient's blood or stock strain agglutinated by the patient's serum are reliable.   Agglutination tests with a freshly isolated autogenous

strain of bacteria are not practical because the organism usually shows marked auto-agglutination.   Complement fixation is satisfactory, although the time elapsing between isolating the organism, making the antigen and performing the test is considerable. The selection of a proper organism from a focus is difficult unless one is equipped to make cataphoresis determination.

Vaccine is prepared in the usual manner, using a non-toxic chemical for killing the organism and making up a stock solution of 1000 million bacteria per cubic centimeter.   From this preparation dilutions for intravenous administration are made so that bottle no.1 contains 1 million per cubic centimeter, no.2, 10 mil-

TABLE 6

COMPLEMENT TITRE

AMOUNT OF SERA CONTAINING ONE UNIT OF COMPLEMENT	
cc.	cc.
0.015	0.005
0.01	0.025
0.05	0.01
0.02	0.02
0.015	0.05
0.025	0.02
0.01	0.05

(The normal range is 0.005 to 0.015.)

lion, and no. 3, 100 million.   The initial dose is 100 thousand bacteria and the average maximum dose 10 million.   Vaccine administered in this way decreases the patient's hypersensitivity and we have found it more satisfactory than the subcutaneous method.

The results of blood counts taken in our rheumatoid arthritis and osteoarthritis cases are shown in table 7, and it will be noted that the percentage of hemoglobin was lower, the number of erythrocytes less and leukocytes somewhat higher in rheumatoid arthritis than in osteoarthritis.

It was found that the blood counts of private cases of rheumatoid were higher in every respect than those from the out-patient department.   There are two explanations for this difference, first,

TABLE 7
Blood Findings in Rheumatoid Arthritis and Osteoarthritis

BLOOD	RHEUMATOID ARTHRITIS (200 CASES)	OSTEOARTHRITIS (79 CASES)
	per cent	per cent
Hemoglobin (Newcomer):		
Over 90 per cent	9	21
80–90 per cent	24.5	50
70–80 per cent	34	23
Below 70 per cent	32.5	6
Erythrocytes:		
Over 4.5 millions	37	51
4.0–4.5 millions	37	33
3.5–4 millions	31	15
Below 3.5 millions	5	1
Leucocytes:		
Below 5,000	4	6
5–10,000	68	80
Over 10,000	29	14

TABLE 8
Comparative Blood Findings in Private and Clinic Cases of Rheumatoid Arthritis

BLOOD	PRIVATE (100)	CLINIC (100)
	per cent	per cent
Hemoglobin (Newcomer):		
Over 90 per cent	16	2
80–90 per cent	32	17
70–80 per cent	34	34
Below 70 per cent	16	47
Erythrocytes:		
Over 4.5 millions	57	17
4.0–4.5 millions	29	44
3.5–4 millions	11	32
Below 3.5 millions	3	7
Leucocytes:		
Below 5,000	4	2
5–10,000	66	70
Over 10,000	30	28

the poor living conditions of the clinic patients and, second, the higher percentage of advanced cases in this group. A comparative study of the findings in these two groups of cases may be found in table 8.

An analysis of the blood counts of early, established and advanced cases was made in the private group (table 9) and showed that the hemoglobin became lower as the disease ad-

TABLE 9

BLOOD FINDINGS IN VARIOUS STAGES OF RHEUMATOID ARTHRITIS

(100 private patients)

BLOOD	EARLY (44 CASES)	ESTABLISHED (32 CASES)	ADVANCED (24 CASES)
	per cent	per cent	per cent
Hemoglobin (Newcomer):			
Over 90 per cent	19	17	12
80–90 per cent	37	27	32
70–80 per cent	29	37	36
Below 70 per cent	15	19	20
Erythrocytes:			
Over 4.5 millions	55	55	60
4–4.5 millions	30	29	30
3.5–4 millions	12	12	8
Below 3.5 millions	3	4	2
Leucocytes:			
Below 5,000	7	0	4
5–10,000	57	72	70
Over 10,000	36	28	26
Shift to left	28	17	15

vanced and in the early cases a higher leucocyte count and a more frequent shift to the left were found. In some of the advanced cases the granulocytes were persistently below normal.

The blood chemistry in rheumatoid arthritis was essentially negative. In rare instances the uric acid was above normal, apparently associated with a gouty diathesis. Only one case showed a low calcium-phosphorus index. At the present time there is but little known about the bone pathology in rheumatoid

arthritis.   We have observed marked decalcification in the x-ray films of some cases, increased density in other clinically similar cases, and degenerative changes in a few of the advanced group, but in all of these cases a normal amount of calcium and phosphorus was found in the blood.   Perhaps further studies of the parathyroid and the vitamins in their relation to bone physiology and pathology will throw light on this phase of the subject.

Gonococcus fixation tests were made as a routine in our cases and in rare instances the results were 4 plus.   We have no way of determining the significance of this test in chronic infectious polyarthritis because in our positive cases there was also evidence of streptococcal infection.

The stool examinations frequently revealed evidence of faulty digestion and elimination.   Thirty per cent showed the presence of streptococci usually of the alpha or gamma types.   Occasionally a hemolytic colon bacillus was found and in two cases the patient's serum showed complement fixing antibodies for this organism.

The metabolic rate was determined in forty-seven of the 200 cases of rheumatoid arthritis.   There were minus readings in 53 per cent, averaging -7, and plus readings in 47 per cent, averaging $+14$.   As the disease advanced the minus readings increased as indicated by 42 per cent, average $+5$, in early cases, and 64 per cent, average -9, in the more advanced cases.   In two cases hyperthyroidism requiring thyroidectomy developed quite suddenly more than a year after the onset.

In conclusion, it is obvious that the laboratory, particularly in the fields of bacteriology and serology, is solving many of the problems of rheumatoid arthritis.   As we learn more about the natural and acquired susceptibility and resistance of joint tissues, and more about the patients immunological and allergic reactions to focal, systemic and joint infections, the treatment of this dreaded disease becomes less emperic and more specific.

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CHOLESTEROLURIA IN BRIGHT'S DISEASE*†

A Chemical Study

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As early as 1863, Salisbury[24] published a paper on "Experiments Connected with the Discovery of Cholesterine and Seroline, etc." Cholesterol crystals were observed in the urines of seventeen patients with a variety of diseases including intermittent fever, typhoid fever, diptheria and chicken pox. In two normal urines examined by Salisbury, no cholesterol crystals were found. The advent of the polarizing microscope revealed a new means of detecting the esters of cholesterol in urine. A number of workers were unable to find anisotropic or double refractile bodies (cholesterol esters) in normal urine (von Noorden[20], Munk[19], Gross[12], Miloslavich[16]). By chemical means, however, the urine from normal subjects was found to contain traces of cholesterol (Pribram[22], Gérard[11], Bacmeister and Havers[2], Grunke,[13] Gardner and Gainsborough[10], Mirsky[17]). In Bright's disease, double refractile bodies were consistently found in the urine (Kaiserling and Orgler[14], Munk, Kollert and Finger[15], Beale[4], Bergel[5], Miloslavich, Brice[6], Achard[1]). Anisotropic substances have been found in the urine in cases of nephritis complicated by diabetes (Falk and von Siebenrock[9]), in nephritis associated with syphilis (Stengel and Austin[29]), and in the albuminuria of pregnancy (Eufinger[8]). Miloslavich quotes the interesting work of Weltmann and Biach and of Genck who found cholesterol in the urine in experimental hypercholesterolemia only after the kidneys had been damaged with uranium nitrate.

The quantitative determination of cholesterol in the urine in Bright's disease, however, has received comparatively little attention.   The literature contains a number of reports on the chemical determination of the urinary cholesterol in one or more cases of renal disease (Bauman and Hansmann[3], Schmidt,[25] Gross, Grunke, Sokolow[28], Mirsky).   A significant work on this subject was published by Gardner and Gainsborough in 1925.   In parenchymatous nephritis with hypercholesterolemia, they found large quantities of cholesterol in the urine approximating 40 mgm. a day.   They maintained that an ethereal sulphate of cholesterol was present in considerable quantities in such urines which could only be determined after drying the ether-extracted urine and boiling the residue with glacial acetic acid under reflux for five to six hours, an observation we have been unable to confirm.   They stated that the high content of ethereal sulphate in the urine goes hand in hand with a high plasma cholesterol.

An investigation of this subject was undertaken in this laboratory three years ago.   The study was chemical in nature; no attempt was made to determine the presence or absence of anisotropic substances in the urine.   Obviously, the quantitative determination of the cholesterol in the urine is to be preferred to a microscopic examination; Brice and Turner[30] have recently pointed out the many difficulties encountered in the recognition of polarizing figures and their identification as cholesterol esters in the urine.

MATERIALS AND METHODS

This report is based on several hundred determinations on the cholesterol and protein content of the urines of thirty-one cases of Bright's disease.   Sixteen of these patients had chronic diffuse glomerular nephritis, five, chronic diffuse glomerular nephritis with a definite nephrotic component, five, with amyloid nephrosis and five, with so-called lipoid nephrosis.   This study was carried out for the most part on cases admitted to the wards of the New York Post-Graduate Hospital.*

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* We are indebted to Dr. S. Edward King for permission to study the five cases of amyloid nephrosis from his service at Sea View Hospital, Staten Island, and to Dr. Carl H. Greene for two cases of lipoid nephrosis from his service at St. John's Hospital, Brooklyn.

The determination of cholesterol in the urine was carried out according to procedures developed in this laboratory. The method described by Mirsky has been adopted with several modifications for urines with comparatively low cholesterol content. The determination of cholesterol in urines rich in this sterol was made by a modified Bloor method. The modifications for both methods referred to above will be described elsewhere. The analyses were carried out on filtered urine; in those circumstances where unfiltered urine was examined they are so indicated in the tables. When the plasma cholesterol was also determined, the Sackett modification of the Bloor method[23] was employed, using the temperature control procedure adopted in this laboratory.[18] The protein content of the urine was determined according to the method of Wu and Ling[31] as described by Peters and Van Slyke.[21]

RESULTS

The concentration and total output of cholesterol in eight urines obtained from six normal subjects are shown in table 1. It is obvious that the concentration of cholesterol in normal urine is exceedingly small, but the quantity present is measurable chemically, especially when quantities of 1000 cc. or more of urine are used for a single determination. The output of cholesterol in normal urine averages 0.5 mgm. in 24 hours; the highest value obtained was 1.1 mgm. for filtered urine and 2.1 mgm. for unfiltered urine per day. The difference between the cholesterol content of filtered and unfiltered urine in one case indicated that the sediment contained approximately 47.4 per cent of the total urinary cholesterol (see footnote table 1).

The relation between the cholesterol and protein concentrations in the urine of a case of chronic diffuse glomerular nephritis with a marked nephrotic component is charted in figure 1. In this study the urine was collected over four hour periods at intervals for twenty-eight days. At the two-hour mark of each of the experimental observations, a blood specimen was obtained and the plasma cholesterol determined. This figure indicates the following significant findings: (A) The excretion of cholesterol in the urine parallels the protein excretion. (B) The level of the blood cholesterol influences the excretion of cholesterol in the urine only to this extent, that more cholesterol is excreted per gram of protein in the presence of a higher blood cholesterol than when the blood cholesterol is at a lower level. Hypercholesterol-

emia, per se, does not induce cholesteroluria (as indicated in studies of the urinary cholesterol in other diseases accompanied by hypercholesterolemia, namely, diabetes mellitus); in the presence of proteinuria, however, an increased blood cholesterol augments the cholesterol excretion in the urine.

The relation between the protein and cholesterol concentrations in the urine of a patient with lipoid nephrosis is given in figure 2. The same relationship between protein and cholesterol excretion described above is indicated in the study of this case.

TABLE 1

THE CHOLESTEROL CONTENT OF THE URINE OF NORMAL SUBJECTS

NUMBER	VOLUME†	TOTAL CHOLESTEROL	
	cc.	mgm. per cent	mgm.
1	900	0.036	0.329
2 A	3,160	0.019	0.600
B	3,020	0.012	0.365
C	2,625	0.042	1.110*
3	1,046	0.006	0.065
4	2,192	Very faint trace	
5	2,070	0.010	0.205
6	1,500	0.068	1.020

* Unfiltered urine in this case showed a concentration of 0.079 mgm. of cholesterol per 100 cc. The total excretion was 2.11 mgm. for the 24 hours. Hence the urinary sediment contained 47.4 per cent of total urinary cholesterol.

† All were 24 hour specimens except number 4 which was a 29 hour speciman.

Figure 3 is presented to show the terminal drop both in protein and cholesterol excretion in the urine prior to death of a patient with nephrotic glomerular nephritis. Exitus was brought about by pneumococcic peritonitis which developed during the course of the disease and not by renal insufficiency.

Table 2 indicates the extent of loss of cholesterol and protein in the urine in patients with Bright's disease. The maximal excretion of cholesterol in the urine of the thirty-one cases studied was shown by the first patient (Case 7) who had chronic diffuse glomerular nephritis with a nephrotic component. This patient excreted 92 mgm. of cholesterol and 50.3 grams of protein in the urine during a period of 24 hours. In contrast to the first four

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cases presenting the nephrotic syndrome, the other seven with chronic diffuse glomerular nephritis showed a much smaller excretion both of cholesterol and of protein in the urine.
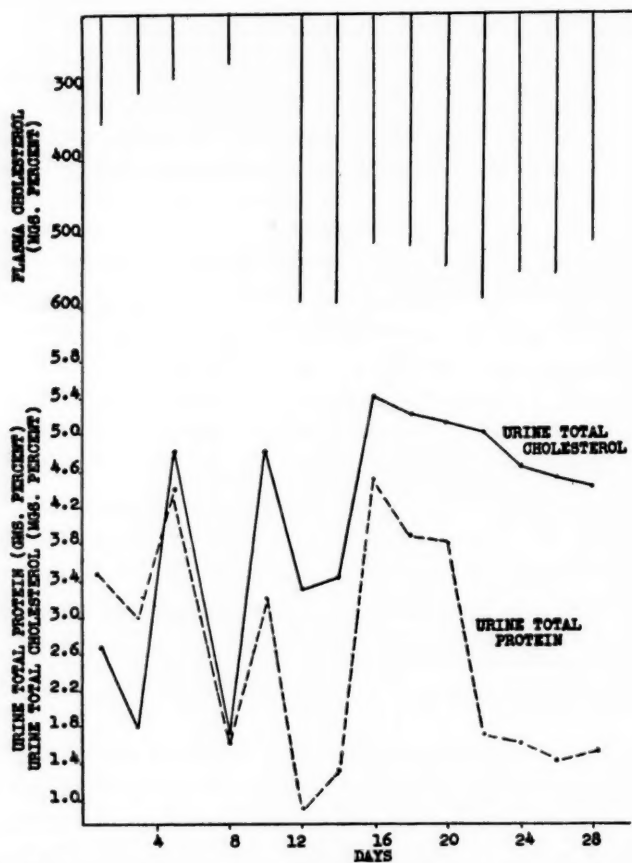
FIG. 1. THE RELATION BETWEEN THE CHOLESTEROL AND PROTEIN CONCENTRATIONS IN THE URINE OF A PATIENT WITH CHRONIC DIFFUSE GLOMERULAR NEPHRITIS SHOWING A MARKED NEPHROTIC COMPONENT (CASE 7)

The effect of variations in the plasma cholesterol on the concentration of cholesterol in the urine

Studies were carried out in an attempt to determine the quantity of cholesterol present in the sediment of nephritic and nephrotic urine (figure 4). This was determined by analyzing

filtered and unfiltered specimens of the same urine, the difference representing the cholesterol in the sediment.   The cholesterol
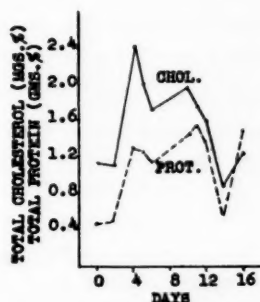
FIG. 2. THE RELATION BETWEEN THE PROTEIN AND CHOLESTEROL CONCENTRA-
TIONS IN THE URINE OF A PATIENT WITH LIPOID NEPHROSIS (CASE 9)
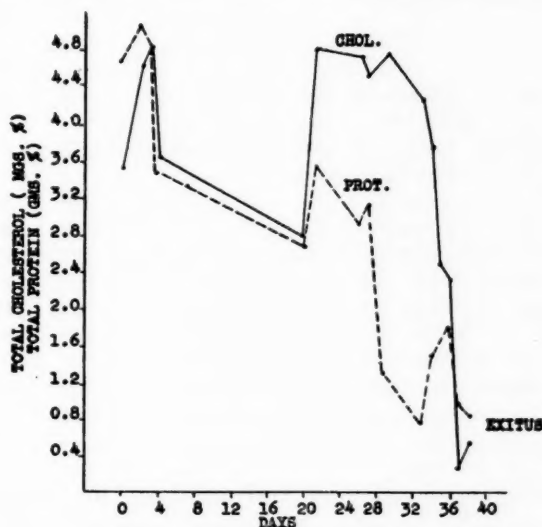
FIG. 3. THE RELATION BETWEEN THE PROTEIN AND CHOLESTEROL CONCENTRA-
TIONS IN THE URINE OF A PATIENT WITH CHRONIC DIFFUSE GLOMERULAR
NEPHRITIS SHOWING A MARKED NEPHROTIC COMPONENT (CASE 7)

Note the fall in the urinary protein and cholesterol several days prior to death from pneumococcic peritonitis.

content of the sediment may at times constitute as much as 40 per cent of the total urinary cholesterol (figure 4).   At times, the

sediment may be comparatively rich in free cholesterol and poor in esters and vice versa (figure 4).

Examples of three of the many experiments carried out on the sediment of pathological urines are summarized as follows: In experiment 1, performed on a patient with lipoid nephrosis the difference between unfiltered and filtered urine indicated that 0.630 mgm. of cholesterol was present in the sediment of 100 cc. of urine. In this case, the sediment of 100 cc. of urine was ob-

TABLE 2

The Extent of Loss of Cholesterol and Protein in the Urine of Patients with Bright's Disease

CASE	DIAGNOSIS	URINE		
		Output, 24 hours	Total cholesterol output, 24 hours	Total protein output, 24 hours
		cc.	*mgm.*	*grams*
7	Chronic diffuse glomerular nephritis with nephrotic component	2,300	92.00	50.32
8	Chronic diffuse glomerular nephritis with nephrotic component	3,270	57.60	22.21
9	Lipoid nephrosis	2,132	34.37	30.50
10	Lipoid nephrosis	546	30.19	25.06
11	Chronic diffuse glomerular nephritis	2,190	21.46	10.51
12	Chronic diffuse glomerular nephritis	1,780	18.08	5.64
13	Chronic diffuse glomerular nephritis	592	11.13	9.89
14	Chronic diffuse glomerular nephritis	2,047	10.06	8.78
15	Chronic diffuse glomerular nephritis	1,422	9.60	4.38
16	Chronic diffuse glomerular nephritis	657	3.68	2.40
17	Chronic diffuse glomerular nephritis	414	2.54	4.86

tained by centrifugalization and analyzed for cholesterol. The quantity actually found, 0.618 mgm., compares favorably with the amount obtained by the difference between filtered and unfiltered urine. In experiment 2, on a patient with chronic diffuse glomerular nephritis with a nephrotic component, the sediment of 1000 cc. of urine was obtained by centrifugalization and analyzed for cholesterol. In this instance, 0.072 mgm. of cholesterol was present in the sediment of 100 cc. of urine. Obviously, the urines of patients with Bright's disease, not only contain varying

amounts (and kind) of sediment, but the quantity of cholesterol present varies considerably in different cases.   No attempt was made to weigh the sediment in order to determine the amount of
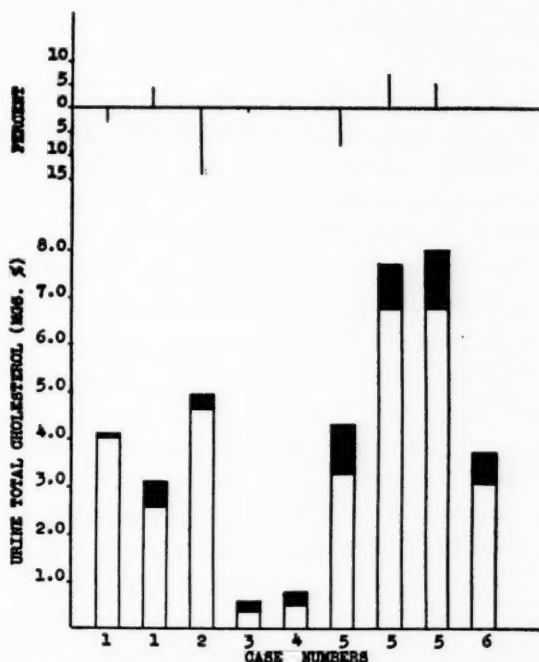
FIG. 4. THE CHOLESTEROL CONTENT OF THE URINARY SEDIMENT OF PATIENTS WITH BRIGHT'S DISEASE

The solid areas represent the difference between the urinary cholesterol concentrations of nine filtered and unfiltered specimens of urine from six patients with Bright's Disease (total column-concentration of cholesterol in unfiltered urine; blank column-concentration of cholesterol in filtered urine).   The solid areas therefore indicate the cholesterol content of the urinary sediment.   The percent increase or decrease of cholesterol esters in filtered urine in comparison to the unfiltered specimens is shown by the lines above and below the horizontal line at the top of the figure.

cholesterol present per unit of weight; this appears to be an interesting problem and it is planned to investigate this in the near future.   In experiment 3 similar studies were carried out for cholesterol and for protein.   The sediment of this urine contained

14 per cent of the total cholesterol (1.168 mgm. per 100 cc.) and only 3 per cent (0.088 mgm. per 100 cc.) of the total protein. Many observations of this type have been made and the results invariably showed that the urinary sediment contained considerably more cholesterol than protein.

DISCUSSION

The most significant finding in this investigation has been the observation that the cholesterol excretion in the urine has been found to parallel the protein excretion. In patients with nephrosis and nephrotic glomerular nephritis, a marked loss of protein in the urine is accompanied by an excessive excretion of cholesterol. The highest output we have observed was a loss of 92 mgm. of cholesterol in 24 hours. At this rate of excretion, and providing the cholesterol in the blood is not replenished, the total blood cholesterol can be depleted within a few months. Obviously, there are processes which compensate for such a marked loss of cholesterol from the organism.

In recent years, much evidence has accumulated to show that cholesterol can be synthesized and destroyed in the animal body. Schoenheimer and Breusch[27] recently reviewed the literature on this subject and also reported the results of experiments on mice which prove that cholesterol synthesis and destruction continually take place in the animal organism. Schoenheimer[26] also established the probability of cholesterol destruction in a patient with hypercholesterolemia.

In view of these observations, it is reasonable to assume that the blood cholesterol is maintained when hypercholesteroluria exists by one or more of such compensatory processes as: increased cholesterol synthesis, decreased cholesterol destruction or diminished excretion through the intestines. A similar thesis to account for the conservation of cholesterol in the organism in other pathological conditions associated with marked losses of cholesterol has been proposed (Bruger[7]). It is impossible to state at this time which of these variables is particularly influenced in morbid states associated with abnormal excretions of this sterol from the body.

CONCLUSIONS

(1) Cholesterol is excreted normally in the urine in amounts averaging 0.5 mgm. per day.   In patients with Bright's disease, the maximal excretion observed was 92 mgm. per day.

(2) In the urine of patients with Bright's disease, the cholesterol excretion was found to parallel the protein excretion.

(3) Hypercholesterolemia, per se, does not induce cholesteroluria; however, when proteinuria exists, more cholesterol is excreted per gram of protein in the presence of a higher blood cholesterol than when the blood cholesterol is at a lower level.

(4) The urinary sediment may contain as much as 40 per cent of the total cholesterol in the urine.

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PLASMA CHOLESTEROL CONCENTRATION IN GLO-MERULONEPHRITIS AND OTHER TERMINAL STATES*

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The wealth of data available regarding alterations in blood cholesterol in pathological states is in striking contrast to the lack of specific knowledge of the mechanisms underlying these changes. This is, of course, due to the relatively limited state of our present understanding of the intermediary metabolism of cholesterol. Inability to evaluate adequately all of the factors which may be operating has unquestionably been responsible for many of the variable and apparently contradictory observations reported by different investigators in a number of clinical conditions. A more complete understanding of the pathologic physiology in certain disease states has clarified the situation considerably, as in the case of renal disease, in which a clearer distinction between predominantly degenerative tubular lesions and inflammatory glomerular lesions has to a large degree explained much of the discrepancy in the earlier reports of blood cholesterol concentration in Bright's disease.

The rather constant occurrence of hypercholesterolemia in chronic nephrosis and in association with advanced nephrotic lesions in glomerulonephritis is well recognized, although its cause is not definitely known. However, whatever the underlying mechanism may be, it is in all probability quite different from that responsible for the alterations in cholesterolemia which may occur in chronic glomerulonephritis, with minimal tubular lesions. Although the total fat, fatty acid and phospholipid con-

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centrations in the plasma are commonly increased, the plasma cholesterol is frequently normal or subnormal in the latter condition, this dissociation arguing, as stated by Peters and Van Slyke,[13] a functional differentiation between these lipid fractions.

The variability of the plasma cholesterol in chronic glomerulonephritis has attracted considerable attention, practically all investigators of this problem coinciding in the opinion that a fall from a previously elevated or normal level is of serious prognostic significance, particularly if associated with increasing nitrogen retention.   This view has been suggested if not actually stated by Chauffard, LaRoche and Grigaut, Schmidt, Epstein and Rothschild, Stepp, Henes, Fleming and Ashe and Bruger.[1] Hypocholesterolemia in advanced nephritis has also been reported by Bloor,[3] Daniels[5] and Kimura and Stepp,[8] but, in contrast to several of the authors mentioned above, Daniels and Hahn and Wolff[7] could not demonstrate an inverse relationship between the blood non-protein nitrogen and cholesterol in this condition. Ashe and Bruger state that whereas there is some evidence of a reciprocal relation between these two factors, at times this is offset by other factors.   Critical analysis of available data apparently supports the conclusion reached by these authors, namely, that hypocholesterolemia in the terminal stages of chronic glomerulonephritis with renal failure is probably due in large measure to contributing factors such as anemia, starvation and cachexia.   As is well known, these factors may in themselves produce or, at least, be accompanied by a fall in plasma cholesterol and, not infrequently, a rise in fatty acids and phospholipid, constituting a blood lipid picture closely resembling that encountered in uremia.

Material and methods

The data reported in the present study consist of observations made in eighteen patients (fourteen of whom died) with advanced chronic glomerulonephritis, thirty-two patients with nitrogen retention not dependent upon glomerulonephritis (26 of whom died) and eighteen patients dying of conditions not accompanied by nitrogen retention.   Plasma cholesterol was determined by the method of Myers and Wardell, urea nitrogen by the aeration method of Myers, non-protein nitrogen and creatinine by the colorimetric methods of Folin and

TABLE 1
CHRONIC GLOMERULONEPHRITIS

CASE	AGE	BLOOD PRESSURE	DATE	CHOLESTEROL	NON-PROTEIN NITROGEN	CREATININ	UREA CLEARANCE	CO_2 CAPACITY	URINE Alb.	URINE Sp. Gr.	HEMOGLOBIN	ERYTHROCYTES	DATE OF DEATH OR DISCHARGE
				mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	per cent	per cent			per cent	millions	
1	23	204/108	3/22/34	59	125	12.1	5	16.6	++++	1,014	48	2.8	3/26/34, aplasia right kidney
			3/24/34		341	19.6		12.8					
			3/25/34		349	20.2		15.7					
2	48	134/80	8/23/34	92	148	11.8		24	++	1,014	68	3.7	9/1/34, myocardial failure
			8/27/34		188	14.4							
			8/29/34	84	253	15							
3	47	170/98	4/ 5/34	157	55	2.2	28		++	1,020	65	3.0	5/9/34, diabetic gangrene. Blood sugar 310 mgm.
			4/18/34		77	3.5							
			4/23/34		64	2.1							
			4/30/34		74	2.5		14.7					
			5/ 8/34	110	117								
			5/ 9/34		227	10.5	6						
4	42	174/102	7/12/34	99	151	15.8			+++	1,012	42	2.0	7/16/34, myocardial failure
			7/13/34	123	207	16.6							
			7/16/34	179									
5	52	170/100	3/23/34	142	92	4.2	14		++	1,010	50	2.7	Discharged, 4/7/34
			4/ 3/34		146	18.4							
			4/ 4/34		184	18.4							
			4/ 7/34	135	193	19.6							
6	38	134/80	8/17/34	83	120	6.1	8		+	1,006	54	3.2	8/22/34, myocardial failure
			8/20/34	80	192	7.8		34					

No.	Age	Blood pressure	Date										Remarks
7	32	240/156	10/ 9/34	100	44	2.5	16		+	1,020	70	3.4	12/16/34
			12/ 7/34		110	7.6							
			12/14/34		156	12.6							
8	35	272/148	11/ 9/33	119	98	4.9			++	1,020	60	3.6	12/22/33, hyperthyroidism
			12/ 1/33		123	7.9							
			12/18/33		150	10.5							
9	37	162/112	12/22/34	104	150	6.2			++	1,022	68	3.8	Discharged, 1/5/35
			12/29/34	136	60	1.5							
10	35	188/96	4/ 3/34	123	136	13.3		21.4	+++	1,014	40	3.3	4/6/34
11	18	200/136	1/ 5/34	168	135	5.9	5	22	++	1,017	35	1.7	Discharged, 3/28/34
			3/22/34	348	91	7.5	7.5	24					
12	41	231/120	8/ 4/34	116	87	4.8	3		++	1,018	63	4.1	8/10/34
				107	126	8.7							
13	52	85/50	9/26/34	84	94	3.4			+	1,013	10	1.7	9/26/34, bleeding ulcer
14	40	210/110	9/10/34	79	90	5.1			+	1,025	78	5.2	9/10/34, myocardial failure
15	27	185/110	12/ 4/34	139	90	6.5	9		++	1,016	48	3.1	12/19/34
			12/11/34	137	71	4.6							
16	34	175/100	1/11/35	153	75	2.8	10		++	1,018	62	3.5	1/28/35
17	38	224/140	11/12/34	195	63	1.4	8	59	++	1,015	85	4.5	11/25/34
18	41	210/122	1/ 2/35	192	59	2.6	36	57	++	1,021	75	4.0	Discharged, 1/7/35

the CO_2 combining power by the manometric method of Van Slyke.   In our experience, the normal range of plasma cholesterol, by the method employed, is 140 to 200 mgm. per 100 cc.   The diagnosis of glomerulonephritis was established by a careful analysis of clinical and necropsy findings.   Patients with nephrosclerosis with and without renal functional impairment were not included in the nephritic group because of the not infrequent absence of anemia in such cases.

ANALYSIS OF DATA

Chronic glomerulonephritis

There were eighteen patients in this group; fourteen died while under observation, two left the hospital in a critical condition and two were discharged considerably improved clinically.   The findings are presented in detail in table 1. Edema, of moderate degree, was present in only two of the four patients with myocardial failure which was predominantly of the left ventricular type in each instance.   The figures reported for specific gravity of the urine represent in each case the maximum value obtained, and those for blood pressure, hemoglobin and erythrocytes represent the mean of several values obtained during the period of observation (table 1).

The non-protein nitrogen ranged from 44 to 349 mgm. per cent and the plasma cholesterol from 59 to 348 mgm. per cent.   Complicating conditions were present in cases 5 (carcinoma), 8 (hyperthyroidism), and 13 (shock and hemorrhage) which might in themselves be associated with a tendency toward hypocholesterolemia.   It is interesting to note the absence of hypercholesterolemia in case 3 (diabetes mellitus) with a blood sugar of 310 mgm. per cent and with gangrene of several toes of both feet.   Anemia was present in every instance, but there was no consistent quantitative relationship between the degree of anemia and of hypocholesterolemia.   Neither was there any consistency in the relationship between the plasma cholesterol and the non-protein nitrogen or creatinine concentrations.   In general, however, although low values were obtained at all levels of non-protein nitrogen, a cholesterol concentration above 140 mgm. per cent was obtained in only one (case 11) of the fourteen determinations in which the corresponding nitrogen values were 100 mgm. or more per cent.   In contrast to this, cholesterol values of more than 140 mgm. were obtained in seven of the fourteen determinations in which the corresponding nitrogen values were below 100 mgm. per cent.   This tendency toward fixation of the cholesterol level at a relatively low figure as the degree of renal functional impairment increases is illustrated in figure 1.   A rise in cholesterol occurred coincidently with a fall in non-protein nitrogen in cases 11 and 15 and a drop in the former accompanied a rise in the latter in cases 2, 3, 4, 8 and 12.

Acidosis of moderate to severe grade was present in seven of the nine cases in which the CO_2 capacity of the blood plasma was determined.   An advanced grade of renal functional impairment was indicated by the urea clearance test

in ten of eleven cases and relatively marked impairment of concentrating ability was indicated by the relatively low maximum specific gravity in four of the seven cases in which clearance values were not obtained.   The total serum protein concentration ranged from 5.82 to 8.14 grams per 100 cc. in the eight cases in
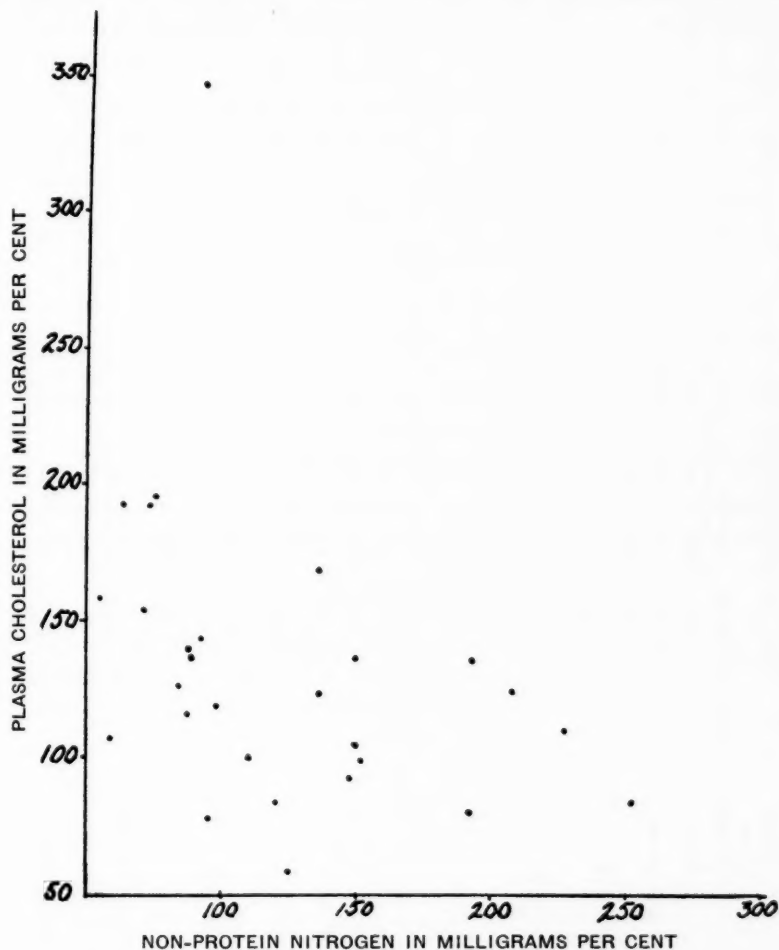

FIG. 1.   GLOMERULONEPHRITIS

which this determination was made, and edema, limited to the lower extremities, was present in only two instances (cases 6 and 14; serum protein 5.82 and 6.76 grams per 100 cc. respectively).   The diagnosis of chronic glomerulonephritis was confirmed at necropsy in ten of the fourteen fatal cases.

TABLE 2
Non-nephritic Nitrogen Retention

CASE	AGE	CONDITION	DATE	CHOLESTEROL	NON-PROTEIN NITROGEN	CREATININ	URINE Alb.	URINE Sp. Gr.	HEMOGLOBIN	ERYTHROCYTES	DATE OF DEATH OR DISCHARGE
				mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.			per cent	millions	
19	67	Prostatism	8/17/34	80	224	9.6			100	5.1	8/18/34
			8/18/34	98	234	9.6					
20	73	Carcinoma of bladder	7/17/34	114	180	18.4	++++	1,034	69	3.5	7/21/34
21	50	Renal calculus	10/ 2/34		24						Well, 11/10/34
			10/23/34	118	187	12.3	+	1,026	94	4.7	
			10/30/34	91	94	6.8					
			11/ 7/34	142	44	1.8					
22	57	Carcinoma of prostate	1/ 8/34		49	2.1	++	1,020			1/19/34
			1/19/34	51	175	6.2					
23	55	Carcinoma of uterus	4/16/34	100	130	16.4	++++	1,012	64	3.4	4/21/34
			4/20/34		161	17.6					
24	70	Prostatism	12/10/34	146	112	4.9	+	1,030	85	4.1	12/22/34
			12/17/34	108	174	8.8					
			12/22/34	113	146	4.4					
25	38	Septicemia	7/19/34	119	33		++++	1,012	53	3.3	7/19/34
26	46	Prostatism	11/ 1/34	98	145	2.4	+	1,017			12/2/34
			11/19/34	89	46	2.1					
			11/27/34	59							
			12/ 2/34								
27	21	Lymphosarcoma	11/22/33	164	57	1.7	+	1,022	65	3.6	12/29/33
			12/29/33		123	3.9					
28	45	Pneumonia	12/13/33	67	120	4.2	+	1,010	61	3.6	12/16/33
29	52	Nephrosclerosis	10/17/34	276	54	2.3	+++	1,022	102	5.1	10/22/34
			10/19/34	314	113	5.6					
30	65	Prostatism	7/20/34	117	112	3.9	++	1,024	80	4.1	Well, 9/1/34
			7/21/34		91	2.4					
			8/16/34	184	36						

No.	Age	Diagnosis	Date								Outcome
31	57	Carcinoma of colon	12/ 4/33		45	1.7	+	1,021	80	4.2	1/5/34
			1/ 5/34	62	109	5.1					
32	64	Nephro-sclerosis	12/22/34	113	81	4.4	+++	1,014	84	4.2	12/27/34
			8/27/34		81	2.9					
33	48	Hyperthyroidism	10/ 2/34	121	77	2.9	+	1,010	61	2.8	Discharged
34	63	Carcinoma of uterus	7/ 5/34		78	5.3	+++	1,027	75	3.5	7/5/34
			8/28/34	83	60	2.8					
35	65	Carcinoma of prostate	9/ 4/34	135	77	2.5	+++	1,010	81	5.0	9/24/34
			9/17/34	84	66	3.0					
36	58	Carcinoma of rectum	6/22/34	128	35	2.5	+	1,028	85	4.7	Discharged, 9/12/34
			8/ 6/34	147	75						
			8/ 9/34		29						
37	39	Jejunal ulcer	9/11/34	98	71	1.4	0	1,023	15	1.2	9/23/34
			9/21/34	63	75	2.0					
38	69	Carcinoma of rectum	5/18/34		61	2.8	+	1,014	58	2.8	5/30/34
			5/28/34	59	71	3.5					
39	57	Prostatism	9/ 4/34		70		+	1,025	99	4.9	Well, 9/14/34
			9/11/34	75	29						
40	57	Peritonitis	10/ 8/34	97	70	1.6	++++	1,019	100	5.1	10/9/34
41	49	Myocardial failure	11/12/33		70		+++	1,020			11/12/33
			8/ 9/34	164	28						
42	66	Prostatism	8/18/34	111	69	2.5	++	1,022	62	3.3	8/24/34
			8/23/34		42	1.5					
43	76	Prostatism	7/31/34		42	1.8	++	1,022	72	3.6	8/25/34
			8/14/34	118	66	1.4					
44	42	Nephrosclerosis	7/ 3/34		41	1.7	++	1,017	60	2.9	7/6/34
			7/ 5/34	156	64	2.3					
45	59	Myocardial failure	7/13/34	196	63	1.9	+	1,014	105	5.0	7/15/34
46	51	Carcinoma of lip	8/ 6/34	124	61	1.7	+++	1,020	80	3.4	8/ 9/34
47	76	Nephrosclerosis	6/26/34	96	59	1.4	++	1,012	98	5.0	6/26/34
48	54	Nephrosclerosis	9/21/34	84	51	1.1	++	1,015	100	4.7	9/24/34
49	69	Bacteremia	7/29/34	69	51	1.6	+	1,024			7/30/34
50	62	Prostatism	7/17/34		41	1.2	+	1,022	81	4.2	7/23/34
			7/22/34	61	47	1.4					

Non-glomerulonephritic nitrogen retention

There were thirty-two patients in this group, twenty-seven of whom died while under observation.   The absence of glomerulonephritis was established at necropsy in twenty instances.   The nitrogen retention was associated with
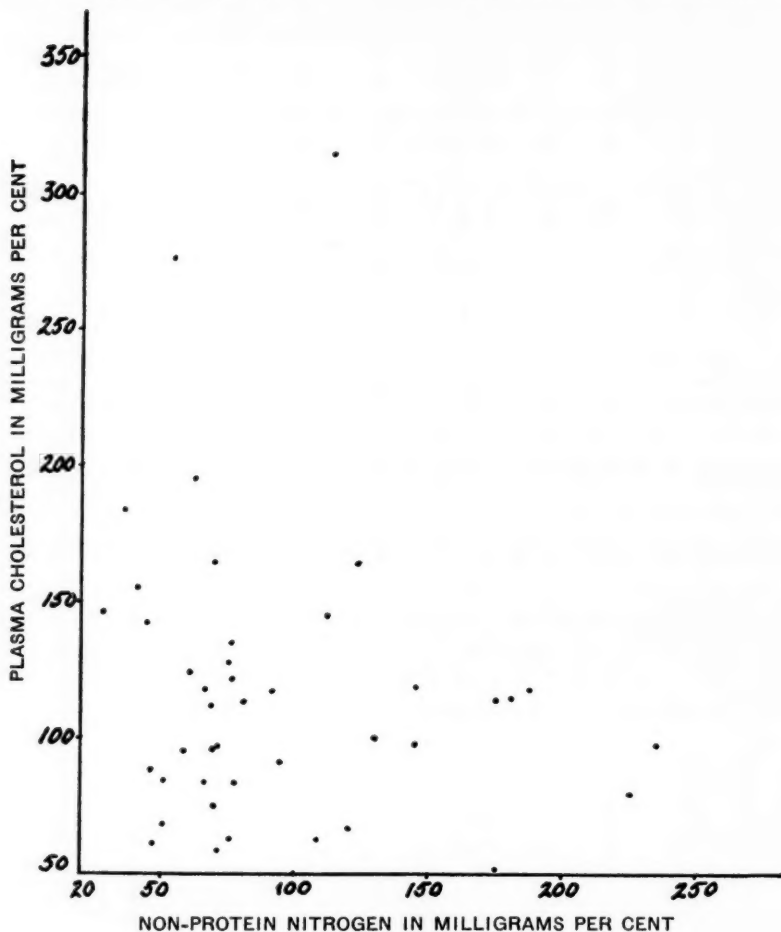

FIG. 2.   NON-NEPHRITIC NITROGEN RETENTION

urinary obstruction in sixteen instances (cases 19, 20, 22–24, 26, 30, 31, 34–36, 38, 39, 42–44), eight of which were due to carcinoma; with nephrosclerosis and hypertension in five; with myocardial failure due to valvular heart disease in two; with renal calculus in one case.   In case 27, the renal tissue was almost entirely replaced by lymphosarcomatous metastases.   Nitrogen retention in the

TABLE 3
TERMINAL STATES WITHOUT NITROGEN RETENTION

CASE	AGE	CONDITION	DATE	CHOLES-TEROL	NON-PROTEIN NITRO-GEN	URINE		HEMO-GLOBIN	ERYTH-ROCYTES	DEATH
						Alb.	Sp. Gr.			
			1954	mgm. per 100 cc.	mgm. per 100 cc.			per cent	millions	1954
51	51	Essential hypertension	10/14	667	39	++++	1,016			10/19, coronary thrombosis
52	28	Hyperthyroid	7/25	98	25					9/11
			8/20	202						
53	86	Arteriosclerosis	7/16	162	38			85	4.3	7/20, pneumonia
			7/18	198						
54	36	Eclampsia	8/3	196	36	0	1,020	71	3.0	8/3
55	46	Carcinoma of liver	7/9	168	29	++	1,025	67	4.7	7/28
56	64	Essential hypertension	10/27	144	38	+++	1,024	98	5.5	10/28, coronary thrombosis
57	43	Essential hypertension	9/24	143	32	+++	1,019	94	5.9	9/24, cerebral hemorrhage
58	34	Congestive heart failure	7/14	134	36	0				7/14
59	39	Carcinoma of esophagus	11/16	133	40		1,020	95	5.6	11/18
			11/17	67						
60	48	Carcinoma of rectum	10/13	122	34	0	1,026	94	4.7	10/14, peritonitis
61	71	Arteriosclerosis	3/19	113	37	+	1,020	78	4.5	3/29, pneumonia
62	67	Bacteremia	8/26	107	24			76	3.7	8/28
63	61	Coronary thrombosis	6/20	106	33					6/20
64	20	Bact. endocarditis	10/2	96	22	+	1,008	69	3.3	10/2
65	54	Essential hypertension	11/22	93	34	+				11/23, heart failure
66	56	Carcinoma of cecum	1/19	79	40	+	1,030	45	3.7	1/20, intestinal obstruction
67	59	Tuberculosis	6/7		34		1,030	80	4.7	7/5
			7/2	77	30					
68	40	Peritonitis	8/1	41	33	+	1,025	87	4.7	8/2

seven other cases was apparently dependent upon primarily extrarenal factors. The non-protein nitrogen ranged from 29 to 234 mgm. and the plasma cholesterol from 59 to 314 mgm. per cent (table 2).

The absence of consistent relationship between anemia and hypocholesterolemia was more apparent in this group than in the patients with chronic glomerulonephritis. As in the latter, there was no consistent quantitative relationship between the plasma cholesterol and the non-protein nitrogen and creatinine concentrations. However, as indicated in figure 2, the distribution of cholesterol values at different levels of nitrogen retention closely resembled that observed in the group of patients with chronic glomerulonephritis, that is, a definite tendency toward fixation of the cholesterol concentration at a relatively low level with increasing grades of nitrogen retention. Values above 120 mgm. were obtained in only three of the seventeen determinations in which the corresponding nitrogen values were 80 mgm. or more per cent, while values above 120 mgm. were obtained in eleven of the twenty-five instances in which the nitrogen values were below 80 mgm. per cent. Decreasing nitrogen retention was accompanied by an increase in cholesterol in cases 21 and 30, both patients recovering. It is interesting to note the progressive fall in cholesterol accompanying a drop in non-protein nitrogen in case 26, in which death occurred on the day of the last recorded determination. Of interest, too, is the high plasma cholesterol (276 mgm.) in case 29, a patient with essential hypertension and nephrosclerosis, rising to 314 mgm. coincidently with a marked increase in non-protein nitrogen.

Terminal states without nitrogen retention

There were eighteen patients in this group, the data being presented in detail in table 3. The plasma cholesterol concentration ranged from 41 to 667 mgm. per cent, the latter value being obtained in a patient with essential hypertension, five days before death due to coronary artery occlusion. No definite cause for this marked degree of hypercholesterolemia could be determined; there was no edema, the blood sugar was 82 mgm. and the total plasma protein 7.4 grams per 100 cc. Values above 140 mgm. per cent were obtained in seven instances, in three of which the determinations were made from five to twenty-one days before death.

DISCUSSION

These observations are in accord with the majority of similar studies reported by previous investigators. The high incidence of low normal and subnormal plasma cholesterol values in the terminal stages of chronic glomerulonephritis and the almost invariable occurrence of hypocholesterolemia in association with high grades of nitrogen retention in this condition lend support to the general opinion regarding the serious prognostic signifi-

cance of such findings in patients with nephritis.   However, the fact that similar findings are obtained in a wide variety of other terminal states accompanied by nitrogen retention of non-nephritic origin suggests that the nephritic process per se may not be the factor directly responsible for the development of the hypocholesterolemia in nephritis and that the prognostic significance of this phenomenon is probably related to the operation of some fundamental mechanism which is stimulated by a variety of pathological states.   Although Bruger and Poindexter[4] found that a sharp drop in plasma cholesterol accompanied an increase in blood urea produced by the oral administration of urea, this reciprocal relation, as stated by Ashe and Bruger, and as illustrated by our findings, is not observed so consistently clinically. The data presented in figures 1 and 2 indicate the frequency of extremely low cholesterol values in association with relatively mild grades of nitrogen retention.   The fact that hypocholesterolemia was present more consistently in patients with marked than in those with mild nitrogen retention does not necessarily, of course, indicate a reciprocal relation between these two factors, but may be dependent upon other phenomena more directly representative of the functional state of the organism as a whole. This is suggested by the frequent occurrence of marked hypocholesterolemia in terminal states unassociated with nitrogen retention or renal disease.

Appreciation of these facts has led other observers (Peters and Van Slyke and Ashe and Bruger) to conclude that the lowered plasma cholesterol in advanced glomerulonephritis is perhaps due to anemia and cachexia.   It is difficult to determine the validity of such an assumption because of the almost invariable anemic and cachectic state of individuals in the terminal stages of this disease.   These factors may be no more directly related to the hypocholesterolemia than is the degree of nitrogen retention. That such is the case, at least with regard to anemia, is suggested by several patients in tables 2 and 3, in whom varying grades of hypocholesterolemia were accompanied by essentially normal hemoglobin and erythrocyte values (table 2: cases 19, 21, 31, 32, 35, 39, 40, 47, 48, 50; Table 3: cases 59, 60, 67, 68).   That many

anemic states may be accompanied by hypocholesterolemia is well established, but here too the underlying mechanism is not well understood, and the substitution of anemia for nitrogen retention as a causative agency does not appear to be entirely justifiable.

Whereas the majority of the patients with advanced glomerulonephritis and those with carcinoma were unquestionably cachectic, this term can scarcely be applied universally to those dying of peritonitis, pneumonia, coronary artery occlusion, congestive and left ventricular heart failure and to certain patients with urinary obstruction due to prostatic enlargement.

If one is to assume that the mechanism underlying the production of hypocholesterolemia in various terminal states is essentially the same in each case, it would appear that some other explanation must be sought. That this phenomenon is dependent upon some disturbance in a fundamentally important mechanism is suggested, at least, by the frequency of its occurrence in terminal states, both acute and chronic, and by its usually grave prognostic significance under such circumstances. Certain observations point to the reticulo-endothelial system as being possibly of significance in this connection. A number of investigators, including Leites,[10] Beumer,[2] Moravek and Cashin[11] and Goebel and Gnoiński[6] have reported a decrease in plasma cholesterol following intravenous injection of various colloidal substances which, as stated by Muller,[12] may be interpreted as indicating that low cholesterol values may be obtained by increasing the functional activity of the reticulo-endothelial system, i.e., stimulating the withdrawal of cholesterol from the blood. According to Leites and Beumer this increased withdrawal may also be dependent upon a change in the colloidal state of the blood.

In the course of our studies of hepatic function in a variety of conditions, a distinct difference was noted in some instances between the plasma cholesterol concentration before and that after the injection of bromsulphalein. Tables 4 and 5 contain a resumé of data obtained in fifteen cases with and twenty-two without dye retention. The dosage employed was two mgm. per kilogram of body weight and the blood was withdrawn for analysis immediately before and 30 minutes after the injection of the dye.

A significant decrease (8.5–41.3 per cent of the original value) occurred in eight of fifteen patients showing dye retention of 5 to 80 per cent and in ten of twenty-two (8.0–30.7 per cent) showing no dye retention.   An increase (21.2–64.6 per cent) occurred in four cases in the first group and in one (8.9) in the second group.   On the basis of the observations of Schellong and Eisler,[15] Saxl and Donath[14] and Klein and Levinson that the halogenated phthaleins are removed from the blood by the

TABLE 4

CHANGE IN PLASMA CHOLESTEROL IN FIFTEEN CASES WITH BROMSULPHALEIN RETENTION THIRTY MINUTES FOLLOWING THE INJECTION OF THE DYE

INCREASED CHOLESTEROL				DECREASED CHOLESTEROL			
Number of cases	Cholesterol	Increase	Dye retention	Number of cases	Cholesterol	Decrease	Dye retention
	mgm. per cent	per cent	per cent		mgm. per cent	per cent	per cent
4	112–380	21.2–64.6	5–80	8	114–238	8.5–41.3	5–80

TABLE 5

CHANGE IN PLASMA CHOLESTEROL IN TWENTY-TWO CASES WITH NO BROMSULPHALEIN RETENTION THIRTY MINUTES FOLLOWING THE INJECTION OF THE DYE

INCREASED CHOLESTEROL			DECREASED CHOLESTEROL		
Number of cases	Cholesterol	Increase	Number of cases	Cholesterol	Decrease
	mgm. per cent	per cent		mgm. per cent	per cent
1	179	8.9	10	79–355	8–30.7

reticulo-endothelial system, these changes in plasma cholesterol may conceivably be dependent upon stimulation or depression, respectively, of the functional activity of the cells of this system, resulting from the administration of the dye.   These observations are presented as additional suggestive evidence both that bromsulphalein is removed from the blood largely by the reticulo-endothelial system and that variations in the functional state of this system may be responsible for, or at least be accompanied by changes in the plasma cholesterol concentration.

Because of the relatively limited state of our knowledge of the intermediary metabolism of cholesterol, no definite statement can be made at the present time regarding the cause of hypocholesterolemia in various terminal states. However, it is possible that the mechanism underlying this phenomenon consists in an increased rate of removal of cholesterol from the blood as a result of a state of increased activity of the reticulo-endothelial system.

SUMMARY

Determinations were made of the plasma cholesterol concentration in eighteen patients (fourteen of whom died) with advanced chronic glomerulonephritis, thirty-two with non-nephritic nitrogen retention (twenty-six of whom died) and eighteen dying of conditions not accompanied by nitrogen retention. Although there was a distinct tendency toward fixation of the cholesterol concentration at a low level with increasing grades of nitrogen retention in both the nephritic and the non-nephritic groups, there was no constant quantitative relationship between the degree of cholesterolemia and of nitrogen retention. Low values were usually obtained in the group of terminal states not associated with nitrogen retention. No constant relationship was noted between the degree of hypocholesterolemia and of anemia.

The fact that similar findings are obtained in a variety of terminal states suggests that the development of hypocholesterolemia and its serious prognostic significance under such circumstances are probably related to the operation of some fundamental mechanism which is stimulated by a variety of pathological states. Certain observations are reported which suggest that excessive withdrawal of cholesterol from the blood as a result of abnormal stimulation of the activity of the reticulo-endothelial system may be of importance in the pathogenesis of this phenomenon.

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HYPERPROTEINEMIA, AUTOHEMAGGLUTINATION AND RENAL INSUFFICIENCY IN MULTIPLE MYELOMA*†

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In a recent article one of us (A. G. F.)[6] summarized the literature concerning some of the unique chemical findings and the queer, abnormal hematological and physical properties of blood from cases of multiple myelomatosis. At that time eighteen cases were found in which hyperproteinemia of 8 to 16 grams of protein per 100 cc. serum or plasma was present, and four cases with proteins from 10.8 to 15 grams were added by the author. Attention was again called to the marked tendency in these cases for the erythrocytes to form marked rouleaux in dry blood smears, and in wet films to aggregate in masses distinguishable with difficulty from clumps made by true isoagglutination. Reimann[13] was the first to clinically correlate these phenomena and to suspect hyperproteinemia, and as a cause for this, multiple myeloma, in a case diagnosed arthritis, in which erythrocytes in blood smears formed tight rouleaux and clumping occurred in the pipette when blood was mixed with Hayem's solution. His impression was correct, for the total blood protein was 10.22 grams per 100 cc. plasma, albumin 0.90 grams, globulin 3.84 grams, fibrinogen 5.48 grams, and the necropsy revealed diffuse myelomatosis. Bönniger[3] had a similar experience with a case showing 13.00 grams total protein. Our first four cases likewise showed excessive rouleaux in dry smears and autohemagglutination into masses

in wet films, giving the clue in three cases, suggesting roentgen-rays and chemical analyses which led to the final diagnosis.

More recently Peters and Eisenman[12] in a study of blood proteins in a large variety of clinical conditions found very high values for total proteins, and especially for globulin, in two cases of myeloma. One of the cases had a general rarefaction of bones suspected of being due to hyperparathyroidism and an anemia with high color index which did not respond to therapy. The association with a very high serum globulin gave a clue to the diagnosis which was verified at necropsy. Only two other cases of malignancy showed high globulin values not due to hemo-concentration. Both were cases of carcinomas with generalized bone metastases, one of which was a case of hypernephroma. Other bone tumor cases, both primary and secondary, showed normal globulin.

Magnus-Levy,[10] who has studied the subject of myeloma most thoroughly, stated that of the cases reported in the literature in which blood protein studies were made, about two-thirds showed 8 per cent protein or over in the serum; globulin was always increased and albumin never increased. He stated rightly that the relative frequency of hyperproteinemia cannot be determined until systematic studies are done on all cases of myeloma. In four recent cases of his own, included in the above, two showed high serum protein, 14.8 and 9.9 grams, and two with values of 7.3 and 6.5 grams respectively. Globulin was markedly increased in the first two, slightly in the third, and not in the fourth. Euglobulin constituted the greatest part of the increase. Magnus-Levy considered the increase of euglobulin to be the chief quantitative change in myeloma blood, on which most of the physical changes depend. He believed that the substance in the blood considered by some authors, especially Jacobson,[8] and Shirer, Duncan and Haden,[14] to be Bence-Jones protein because it precipitated at 56°C., is nothing but euglobulin. He prepared a synthetic serum by dissolving in normal serum washed euglobulin obtained by precipitation resulting from diluting serum from two myeloma cases with 60 volumes of distilled water. The resulting synthetic serum yielded a heavy coagulation on heating to 56°C.

for 30 minutes.   By slowly raising the temperature of a water
bath about 1°C. per minute, a precipitate first appeared at 60
to 62°C., while normal serums showed none until about 70°C. was
reached.   Similar differences were found in blood from patients
with myeloma showing increased euglobulin.   Bence-Jones pro-
tein and fibrinogen, which coagulate at low temperatures, were
ruled out in this experiment.   Furthermore, he stated that in
none of his serums from patients with myeloma has he been able
to demonstrate Bence-Jones protein, although addition of as
little as 0.1 per cent is sufficient for demonstration.   This pro-
tein, even in strongest solutions, was not precipitated by 22 per
cent sodium sulphate, as is euglobulin.

Magnus-Levy believed the increased viscosity of myeloma
blood most probably is due to the euglobulin increase, since the
molecular weight of the various proteins as determined by Sved-
berg and Sjogrens is as follows: Bence-Jones protein 35,000,
albumen 70,000, globulin 105,000 and euglobulin even higher.
He attributed the decreased clotting power and the small amount
of serum expressed from the clot to viscosity.   In several of
Magnus-Levy's cases at post mortem, Rössle found the clots to
be stiff and gelatin like, filling the vessels, and not the retracted
clots seen in the average body, especially those of persons dying
of infection.   Similar findings were observed in all our cases
examined at necropsy.   Magnus-Levy also believed that the
euglobulin increase is the cause of the marked rouleaux formation
described by Reimann, Bönniger and Foord, and seen in two of
his own cases.   Apparently the markedly rapid sedimentation
seen in multiple myeloma cases and reported by Reimann, Bön-
niger, Foord and Johansen[9] especially, is associated with the
increased euglobulin.   The only exception is one case of Magnus-
Levy's, which had a normal blood protein.   Finally Magnus-
Levy reiterated his previous statements that the cases showing
increased globulin in the blood are ones with only small loss of
protein in the urine.   No hyperproteinemia is to be expected if
there is a heavy loss in the form of ordinary urine protein or Bence-
Jones protein.   The study of our own material and the other
cases in the literature show the truth of this statement.

Johansen's case, with one reported by Veil,[15] which were not included in our original paper, and a recent case by Cantarow,[4] should also be included as cases of multiple myeloma with hyperproteinemia.   The former showed 8.9 grams total serum protein, 0.4 grams albumin, and 8.5 grams globulin, Veil's case 12.1 grams protein, 5.1 grams albumin, and 7.0 globulin, and Cantarow's case 11.3 grams total protein, 2.8 grams albumin, 2.9 grams globulin, and 5.6 grams considered to be Bence-Jones protein.

The origin of the renal insufficiency in some cases of multiple myeloma is still in dispute.   The careful studies of Bell[2] show that most of the cases result from plugging of the collecting tubules of the kidneys by casts of Bence-Jones protein, leading to cortical atrophy.   In one case sent him from our first series (case 3) the renal insufficiency seemed to him to be due to plugging of the glomerular capillaries by inspissated protein.   In another case in our first series, similar findings were present, and the suggestion was made that perhaps further decrease in glomerular blood flow may have resulted because of intravascular autohemagglutination.   This was demonstrated in the retinal veins by direct opthalmoscopic study in one case when pressure was applied to the eyeball sufficient to slow the blood flow.   Masses appearing like cayenne pepper were seen trickling slowly through the veins.

The following two cases represent the last two cases of multiple myeloma seen by the authors in the past year, both of which were examined at necropsy.   They were undiagnosed until blood studies revealed marked rouleaux formation in the blood smears, which stimulated further laboratory and roentgenographic studies, which in the first case clinched the diagnosis.   In the second case the only roentgen-rays taken during life, two months before death, failed to show destruction of bone sufficient for a diagnosis, but necropsy showed diffuse disease.

CASE REPORTS

Case 1.   A. F., a white male, aged 57, entered the Garfield Hospital on February 5, 1935, soon after being seen by Dr. A. F. Stelhorn.   During the past six months he had lost 30 pounds in weight and had become so progressively weak that he was bedridden on admission.   Loss of appetite was marked and occasional vomiting had occurred during the past few months.   Several physicians who

had seen him before admission had made the tentative diagnosis of carcinoma of the stomach. There was no complaint of pain in any part of the body. A marked anemia had also developed with associated dyspnea and palpitation on exertion. The only feature of interest in the past history was cauterization with complete destruction of a small carcinoma of the lower lip in 1934.

Physical examination revealed a poorly nourished, anemic adult. Temperature 98°F., pulse 88, respiration 22, blood pressure 110 mm. systolic, 60 mm. diastolic. A scar on the lower lip was free from evidence of recurrence. There was no lymphnode enlargement in the neck or elsewhere. The thyroid was not enlarged. Examination of the chest and abdomen was essentially negative. There were no masses or areas of tenderness in the abdomen; the spleen and liver were not enlarged. Rectal examination showed a slight, soft, uniform enlargement of the prostate. The legs showed no edema. Neurological examination was negative. Pressure over the ribs, sternum and vertebrae produced no pain.

Urinalysis revealed acid reaction, specific gravity of 1.014, serum protein, +, Bence-Jones protein, demonstrated by Exton's and Purdy's methods, heavy trace, sugar, negative. The sediment showed a moderate number of hyaline, finely and coarsely granular casts and enormous numbers of uric acid crystals, some in masses resembling casts. Blood studies on February 7, 1935 showed 4.0 grams of hemoglobin (Newcomer), erythrocytes, 1,140,000; leukocytes, 6,500; differential count in per cent showed 59 neutrophiles, of which 3 were juveniles, 7 were stab forms and 49 were segmented, monocytes 14, lymphocytes 21, myelocytes 1, stem cells 1, abnormal plasma cells (tumor cells) 4. In diluting blood in a pipette with Hayem's solution, marked granule formation resulted, so that a count could not be made. No such effect was found when normal sodium chloride solution was used. In stained smears, marked rouleaux formation was seen, even in the thinnest portions of rapidly dried films (fig. 1A). The erythrocytes on the average were slightly larger than normal, and some showed slight or even moderate pallor. Moderate anisocytosis and slight poikilocytosis were present. Some of the polymorphonuclears showed toxic granulation. The plasma cells mentioned above showed rather immature nuclei. Wet films showed marked, prompt autohemagglutination. In sedimentation tubes, cayenne pepper like granules were found in 5 minutes and complete sedimentation resulted in 20 minutes. The patient's cells were agglutinated typically as Type II (Moss) cells. The patient's serum caused isagglutination of Type III cells and such marked hemagglutination (excessive rouleau) with Type II and IV cells that only with difficulty could the clumps be distinguished from true isoagglutination. At 1 to 2 dilution with saline, the same phenomenon was still present. Bleeding time and clotting time were essentially normal. Only a small amount of serum was obtained from the clot. Chemical analysis on February 7, 1935 showed non-protein nitrogen of 148 mgm., creatinine 5.2 mgm., uric acid 20.0 mgm., calcium 14.2 mgm., phosphorus 5.0 mgm., total protein 18.37 grams, albumen 6.0 grams, globulin 11.9 grams, fibrinogen

0.47 grams per 100 cc. blood. Because of the high values for uric acid, the determination was repeated with small amounts of filtrate and checked several times with the same result. On heating the serum slowly along with a normal control, coagulation began at 65°C. in the patient's serum and at 72°C. in the control. Complete coagulation occurred at 70°C. in the patient's serum and at 80°C. in the normal serum. The coagulation in the patient's tube was very firm and much whiter than that of the normal serum. Bence-Jones protein was considered to be absent. Roentgen-rays, suggested after the blood and urine studies, revealed many very small areas of decreased density in the skull, ribs, and vertebrae, consistent with multiple myeloma.

The patient went rapidly downhill, became very restless, requiring morphine to quiet him, and then went into coma and died on February 10, 1935, five days after admission.

Necropsy was performed 16 hours after death, and arterial embalming had been done before consent for examination was obtained. The principal findings were as follows: In all the ribs, vertebrae and sternum, many small areas of rarefaction, in which gray or gray-pink fleshy tumor was found, were present, the largest 1 cm. across, and a diffuse gray growth was present between most of the trabeculae without much destruction of the bone, but replacing nearly all the marrow. Thinning of the cortex of the ribs was marked enough that the bones could be cut with a heavy knife. The femur marrow showed small patches of red marrow, gray tumor, and much fat. The skull was moderately involved. The kidneys weighed together 420 grams and showed a moderately anemic sectioned surface. The cortex was 6 to 7 mm. thick, the striations still visible. The capsules stripped easily, leaving a smooth, gray-pink opaque surface. The spleen weighed 380 grams, was tense, and the sectioned surface showed a gray-pink, opaque, homogeneous pulp in which the Malpighian bodies and trabeculae were obscured. The liver showed nothing of interest except anemia. The lymph nodes throughout the body were not enlarged and appeared normal grossly. The thyroid and parathyroids were normal. The lungs showed hypostatic hyperemia, edema and fresh bronchopneumonia. No primary tumor was found in any of the internal viscera. The prostate was moderately enlarged and the seat of a moderate adenomatous hyperplasia. There was no evidence of recurrence or metastasis from the lip tumor. The blood clots in the heart, aorta, and large vessels were very firm, gelatin like, and the red cells had settled to the dependent portions.

Histological studies of the tumor in the bones showed it to be a typical plasma cell myeloma, (fig. 3A.) causing nearly complete replacement of marrow and various degrees of destruction of bone. Most of the cells were slightly larger than lymphocytes, and many were so cut that their nuclei were found to be eccentric. Watch-face patterns were characteristic. Nucleoli were prominent. Mitoses were rare. Many similar plasma cells were found in the splenic pulp, causing disruption of the normal architecture. In the liver sinusoids, and in the peripheral spaces, proliferation of plasma cells was quite prominent. The

kidneys showed very little patchy fibrosis, no more than to be expected at the age of the patient. The glomeruli in hematoxylin and eosin stained slides appeared essentially negative, and in slides stained by azocarmine, orange G and aniline blue, according to McGregor's[11] technic, the glomerular basement membranes were normal. No endothelial proliferation was seen. Most of the glomerular tufts were plugged by protein. Granular protein, staining blue by the McGregor technic, was seen in most of the kidney tubules. Only a few casts were seen, and no occlusion of the collecting tubules by casts was found. Most of the convoluted tubules showed a slight dilatation and high grade granular degeneration of their cytoplasm. Minimal arteriosclerosis was found. The parathyroids, which were all sectioned, were normal. In the vessels of all the organs, the serum stained very densely, apparently due to a high protein content, and the edema fluid in the lung was exceptionally dense. No intravascular clumping was noted, but embalming apparently played a rôle in dissipating this phenomenon.

The final summary was: Widespread plasma cell myelomatosis with little destruction of bone; plasma cell (leukemia like) growth in spleen, lymphnodes, liver, adrenals; renal insufficiency due to glomerular obstruction; hypostatic hyperemia, edema and bronchopneumonia of lungs; anemia; hyperproteinemia.

Case 2. W. N., aged 56, entered the Alhambra Hospital on December 22, 1934 under the care of Dr. Wayne Woods, complaining chiefly of malaise, loss of weight, strength and color during the past year, but chiefly in the past few months. Six weeks previously he had had a molar tooth extracted, which was followed by a slow oozing which had never been completely stopped by local applications, either by his dentist or physician. He also complained of marked loss of appetite and dyspnea and palpitation on exertion. He had suffered no pain and had no gastrointestinal or other complaints sufficient to explain his general condition. Nocturia, two to three times nightly, had been present for the last few months. There were no neurological symptoms.

Physical examination revealed a very pale, sick appearing man showing marked evidence of loss of weight. There was no evidence of hemorrhage except for a slight oozing from the site of his extracted molar tooth. The gums showed moderate pyorrhea. General physical examination revealed little else of interest. The heart beat was somewhat weak and rapid, the lungs clear. The abdomen was soft, scaphoid, no tenderness or masses were present, and the liver and spleen were not enlarged as determined by palpation or percussion. The lymphnodes in the palpable groups were not enlarged. There was no evidence of enlargement in the thyroid region. The prostate was slightly uniformly enlarged, but was soft. The rectum was negative. Neurologic examination was negative. There was no edema or icterus. Temperature 97.6°F. to 98.2°F., pulse 100, respiration 18 to 20. A transfusion of 500 cc. blood from a Type IV (Moss) donor was done by multiple syringe method immediately after admission, followed within 20 minutes by a chill and tempera-

ture of 104, which came promptly back to normal. Before the transfusion, the bleeding time was 2.5 minutes and the venous clotting time in a 6 mm. tube was 10 minutes. Further laboratory studies were done two days later.

Two urinalyses showed specific gravity of 1.012 to 1.013, acid reaction, protein, +, sugar, negative, and the sediment showed a few hyaline and finely and coarsely granular casts. No pus or blood was present. Tests for Bence-Jones protein by Exton's and Purdy's sodium chloride, acetic acid and heat methods revealed a trace. Blood count showed hemoglobin 7.8 grams (46.2 per cent Newcomer), erythrocytes 2,900,000, leukocytes 21,000, platelets 180,000, and the differential count in per cent showed 0.5 myeloblast, myelocytes, neutrophilic 0.5, myelocytes, eosinophilic 3.5, juveniles 3.5, stabforms
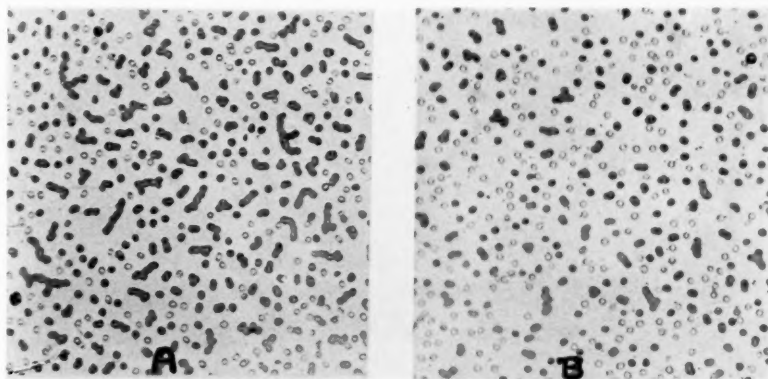

FIG. 1. WRIGHT STAINED SMEARS OF BLOOD SHOWING MARKED ROULEAUX FORMATION

(A) Case 1. (B) Case 2

17.5, segmented neutrophiles 29, eosinophiles 28.5, basophiles 0.5, monocytes 3.5, lymphocytes 13. The erythrocytes on stained smears made quickly and dried by waving in the air showed marked rouleaux formation, even in the thin parts of the smears, (fig. 1B). The individual cells showed a slight achromia and only slight variation in size, with an average diameter slightly less than normal. There were no changes in shape or signs of regeneration. The platelets appeared normal in number and morphology. Some toxic granulation was found in the neutrophiles. Wet smears of oxalated blood showed very marked rouleau formation of the erythrocytes, and within a few minutes large aggregates appeared, leaving very few unclumped erythrocytes. The sedimentation rate was very rapid, clumping of the cells into cayenne pepper like masses occurring within 1 to 2 minutes, and the column of the erythrocytes fell 30 mm. in 5 minutes. The patient's erythrocytes were agglutinated as typical Type II

(Moss) cells.   When the patient's serum, undiluted and diluted 1 to 2 was mixed with suspension of Types II, III and IV cells, a profound hemagglutination occurred with the Type II and IV cells, which was so marked that it could hardly be distinguished from the true isoagglutination occurring with Type III cells, (fig. 2).   At 1 to 4 dilution with saline there was no effect on any type of cell.

Heating the serum for 30 minutes at 56°C. caused no coagulation, nor did any protein precipitate appear as the temperature was slowly raised until 68°C. was reached, at which point a coagulum formed which became very dense as the temperature was elevated more.   A normal serum showed beginning coagula-
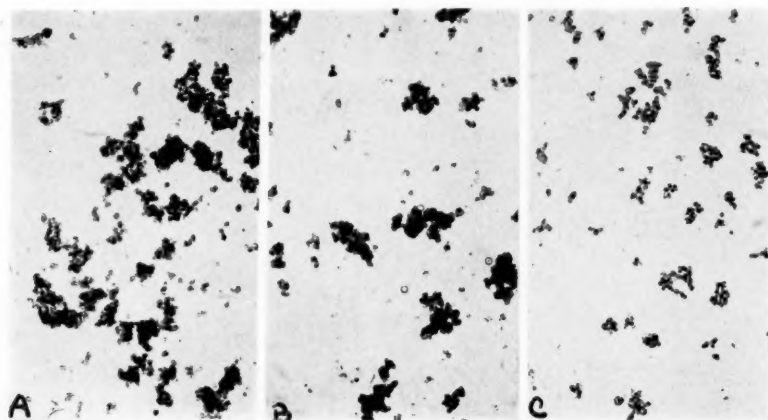

Fig. 2. Case 2

(A) Serum of patient plus Type II cells.   (B) Serum of patient plus Type III cells.   (C) Serum of patient plus Type IV cells.

Note hemagglutination with Types II and IV cells, much resembling iso-agglutination with Type III cells.

tion at 70°C., but the coagulum was not nearly as dense as in the patient's serum.   Bence-Jones protein was considered to be absent.

Blood chemistry showed non-protein nitrogen, 40.6 mgm.; uric acid, 7.0 mgm.; creatinine, 2.0 mgm.; chlorides, 510 mgm. (whole blood), calcium, 9.4 mgm.; phosphorus, 3.9 mgm.; total protein, 12.74 grams; serum albumin, 2.87 grams; serum globulin, 9.38 grams; and fibrin 0.49 gram, per 100 cc. of blood.

Because of the hyperproteinemia and the resulting hemagglutination mentioned above, multiple myeloma was suspected, and roentgenrays of the ribs, vertebral column and the skull were made and were reported as being negative for any evidence of myeloma.

The patient left the hospital after four days and refused medical attention until two months later, when he was taken to the Los Angeles County General

Hospital almost moribund. He had lost much weight and strength and had bled intermittently from the tooth socket ever since two days after the first transfusion. Physical examination revealed no more definite findings than previously. Blood count February 25, 1935 showed 29 per cent hemoglobin (Sahli), erythrocytes, 1,550,000; leukocytes, 131,600; platelets, 71,000, and the differential count in per cent showed 0.5 myeloblasts, premyelocytes 1.5, myelocytes 12, juveniles 11, stabforms 15, segmented neutrophiles 18.5, eosinophiles 31, monocytes 3, lymphocytes 7, and plasma cells 0.5. The leukemoid blood picture was considered to be due perhaps to a preagonal change since the intern found on admission a leukocyte count of 25,000. Marked toxic granulation was found in the neutrophiles. The erythrocytes showed profound rouleaux formation as before. The patient bled more than 20 minutes from the finger, and his venous clotting time was 6 minutes in a small 6 mm. tube. His non-protein nitrogen was 132 mgm., and creatinine 4.6 mgm. per 100 cc. blood. Urinalysis by the intern showed specific gravity of 1.013, protein, negative including Bence-Jones protein, and a negative sugar reaction. He was transfused with blood from a Type II (Moss) donor and suffered a severe chill without evidence of intravascular hemolysis. He continued to go downhill and died 24 hours later.

Necropsy performed 17 hours later by Dr. J. Tragerman and one of the authors (A. G. F.), revealed marked emaciation, clotted blood and necrosis in the molar tooth socket. The bones showed marked changes, chiefly the sternum and ribs and vertebrae. Many nodules of soft gray or gray-pink tumor, the largest about 2 cm. across, and a diffuse growth throughout the marrow spaces, were found in the sternum. Some of the larger nodules had penetrated the internal table and had grown as flat growths on the under surface of the bone. The ribs were likewise affected and could be whittled with a knife. The cortex was thinned, much absorption of trabeculae was present, and nodules from a few millimeters to 1 cm. across were found in addition to a diffuse growth in nearly all the marrow spaces. The vertebrae showed a similar change with only a few areas of red marrow remaining. In the second lumbar vertebra a large nodule 2.5 cm. across was found. The right femur marrow showed a red marrow mixed with an equal portion of fatty yellow marrow. In the skull a few small areas of rarefaction were found.

The kidneys weighed together 300 grams and showed little of interest except occasional small, poorly defined gray areas about 1 cm. across, just under the capsule. The sectioned surfaces were somewhat pale, the markings plainly seen, the parencyhma somewhat swollen. The capsules stripped easily, leaving smooth surfaces. The lungs showed hypostasis, edema, and small areas of fresh pneumonia. The spleen weighed 310 grams and was swollen and tense, the parenchyma was red in color, homogeneous, and the Malpighian bodies were not visible. There was no evidence of lymphnode enlargement. The liver was not remarkable. No tumor was found in any of the other organs. The pituitary, thyroid and parathyroids were normal. The blood clots in the

heart and large vessels were firm, gelatin like, and the red cells had settled in them to the dependent portions.

Histological studies were made of Zenker-fixed tissues stained by hematoxylin and eosin, Giemsa, and azocarmine, orange G, and aniline blue. All the organs showed the serum in the blood vessels to be very dense and in sections stained by McGregor's azocarmine, orange G, and aniline blue combination, the serum was stained orange-red. Similar staining was very marked in the edematous lungs and in the fatty marrow from the femur. The tumor bearing parts of the bone marrow and the larger tumor masses were composed of plasma cells with
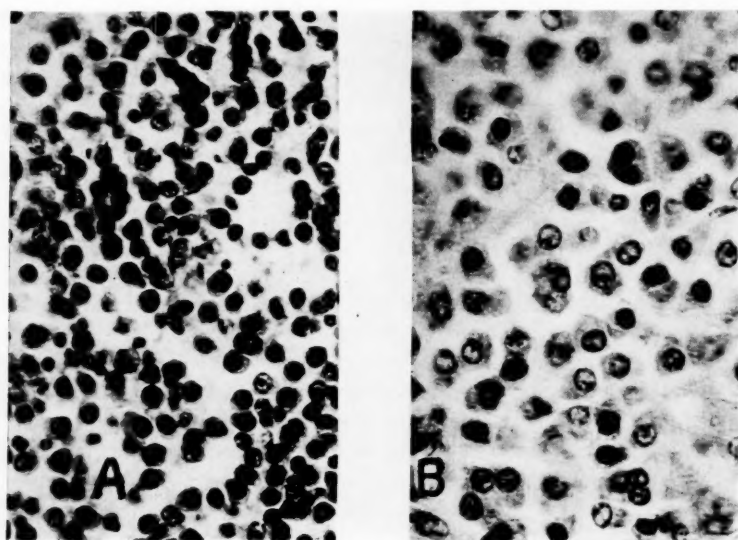
FIG. 3. (A) SECTION OF MYELOMA FROM FEMUR OF CASE 1 SHOWING PLASMA CELLS. (B) SECTION OF MYELOMA FROM FEMUR OF CASE 2 SHOWING PLASMA CELLS

typical round, eccentric nuclei showing watch-face patterns in some, while others were more immature and hyperchromatic, (fig. 3B). There was considerable destruction of bone, but many portions of vertebrae, ribs, and sternum showed a diffuse growth of tumor entirely replacing the marrow, growing in between the non-eroded trabeculae. The remaining islands of marrow in the cancellous bones showed only moderate myeloid proliferation, and, strikingly enough, only a moderate number of eosinophiles and eosinophilic myelocytes. In the femur marrow, large areas of fatty marrow were interspersed between islands of plasma cells and areas of myeloid cells. In the liver, spleen and lymphnodes, plasma cells were found in fairly large numbers and also extramedullary myeloid hema-

topoiesis was found to a rather marked extent.   In all these organs a moderate
number of eosinophiles and eosinophilic myelocytes were found associated with
neutrophilic myelocytes and cells apparently younger than myelocytes, as well
as some erythroblasts.   In the liver the cell accumulations were chiefly about
the portal channels, but also considerable numbers were found in the sinusoids
in all parts of the lobules.   The kidneys showed a few islands of plasma cells,
and in one section a fairly large tumor nodule similar to those in the marrow.
There was no noteworthy fibrosis or dilation of the convoluted tubules, (fig. 4).
Considerable granular debris was found in many of the collecting tubules.   The
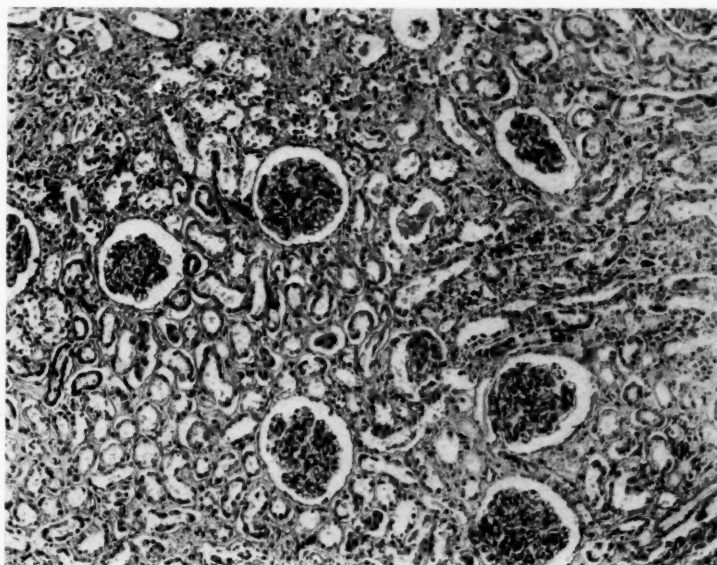
FIG. 4. KIDNEY OF CASE 2.   HEMATOXYLIN AND EOSIN STAIN SHOWING PRACTI-
CALLY NORMAL ARCHITECTURE

glomeruli showed no noteworthy changes in their structure.   Some were col-
lapsed but many showed their capillary loops filled with protein staining orange-
red by the combination stain.   In some of these, clumps of erythrocytes were
found.   In most of the vessels larger than capillaries the erythrocytes were
clumped or arranged in rouleaux similar to the findings in wet films of blood
taken during life (fig. 5).   Similar findings were seen in some of the other organs.
The parathyroids, pituitary, and thyroid were free from noteworthy changes.

Post mortem blood studies showed non-protein nitrogen, 280 mgm.; uric
acid, 15 mgm.; creatinine, 7.0 mgm.; total plasma protein, 16.38 grams; plasma
albumin, 5.3 grams and plasma globulin, including fibrinogen, 11.08 grams per

100 cc. blood. When heated very slowly, coagulation occurred first at 63°C and was complete and very firm at 70°C. A normal control showed beginning and complete coagulation at 73°C. and 80°C. respectively.

The final diagnosis was: Diffuse plasma cell myelomatosis; renal insufficiency due to glomerular obstruction; plasma cell growth in liver, spleen, lymphnodes and kidney; severe anemia; hypostatic hyperemia, edema and bronchopneumonia of lungs; hyperproteinemia.
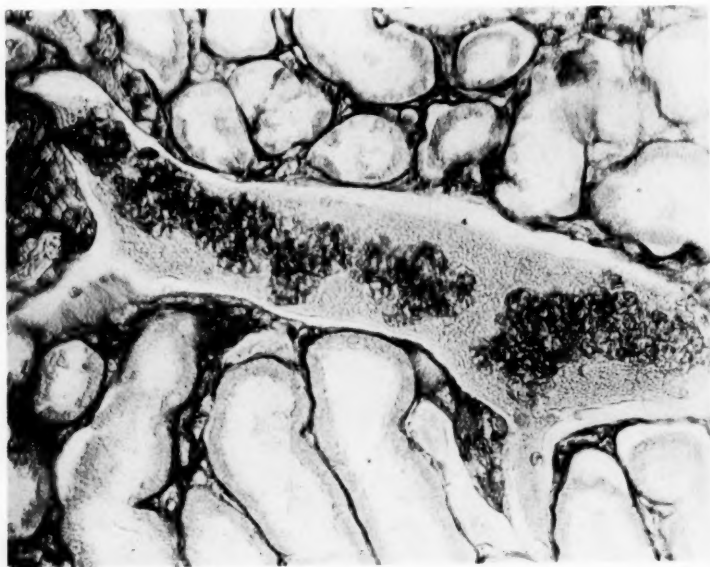
FIG. 5. SECTION OF KIDNEY CASE 2. VEIN SHOWING MARKED AUTOHEMAG-GLUTINATION OF ERYTHROCYTES

DISCUSSION

These two cases demonstrate the fact that multiple myeloma should be always considered in cases of unexplained anemia and loss of weight, and that the finding of marked rouleaux formation in dry smears and excessive autohemagglutination (not to be confused with isoagglutination) in wet smears, should make one consider multiple myeloma as a possible diagnosis. The increased protein demonstrated is similar to the findings in other cases already reported and consisted largely of increased globulin. Separation into euglobulin and pseudoglobulin was not done.

As in other cases in the literature, and especially in the cases seen by us, the hyper inemia occurred with loss of only small amounts of protein in the urine. The tissues of both cases histologically showed increased protein in the blood vessels and in edema fluid in the lungs. The orange-red staining of the protein in the second case in tissues stained by McGregor's technic was particularly striking. The intravascular autohemagglutination found in the dead tissues does not necessarily mean that any such phenomenon was present before death, but previous experience has shown us that merely slowing up the blood stream is sufficient to cause this in the retinal veins. We have not tried microscopy of the capillaries in the nail bed, but Fahraeus[5] stated that in patients with highly increased sedimentation rates, aggregates of erythrocytes can be seen in the capillaries if the blood stream is slowed. We believe that the nitrogen retention in our cases may have been due to plugging of the glomerular capillaries, both by protein in the serum inspissated to form a much more viscid mass because of withdrawal of water by glomerular filtration, and perhaps also by the impediment offered by the aggregated erythrocytes. The same problem comes up in cases of extreme dehydration, as in severe burns. Renal insufficiency here also may be more than a simple toxic effect, and changes in osmotic pressure may play a rôle in the glomerular tuft capillary not accurately measurable by chemical and physical studies of the blood.

The plasma cell growth in the liver, spleen, kidneys and lymphnodes seen in both cases is unusual but has been described by others, especially Jackson, Parker and Bethea,[7] and Barr.[1] The extramedullary myeloid hematopoiesis in case 2 probably resulted from the widespread replacement of marrow by tumor. We have not observed amyloid deposit in the joints or soft tissues about the joints or in the parenchymatous viscera, although Magnus-Levy stated that in thirty-seven out of 150 cases of multiple myeloma examined at necropsy amyloid had been found.

SUMMARY

Two cases of plasma cell multiple myelomatosis, both with widespread involvement of bones and bone marrow and plasma

cell growth in liver, spleen and lymphnodes are presented. One showed extramedullary myeloid hematopoesis. The clue leading to the diagnosis was obtained when blood smears were found to show profound rouleaux formation, and in one case, clumping and precipitation occurred in a pipette when blood was mixed with Hayem's solution. Marked hyperproteinemia and especially hyperglobulinemia was found. This finding has been present in the last six successive cases of myeloma seen by us. The sedimentation rates were profoundly accelerated. Obstruction of glomerular capillaries by protein and the presence of clumps of erythrocytes was noted in the kidney glomeruli. Nitrogen retention may have been caused by this phenomenon. Multiple myeloma should always be suspected in cases of atypical nephritis or in unexplained anemia.

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CONCERNING ANTICOAGULANTS

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The proper anticoagulants to prevent clotting of blood have been the subject of much discussion. Although they have been limited to a few in general use in the laboratory, they are used in various concentrations. The literature is vast but one is struck with the limited amount of critical investigation given to the subject, especially when the anticoagulant may play an important part in calculations based on the hematocrit value. Because of this, the subject has been carefully investigated.

1. ANTICOAGULANTS FOR CHEMICAL DETERMINATIONS*

At present anticoagulants for chemical determinations have been more or less standardized, and their limitations are well recognized. It is rarely necessary to use heparin or hirudin, and because of their expense and variability and because inorganic salts will serve as well, they have not had wide usage. Defibrination may be desirable for some determinations and defibrination or heparin† is best employed when studies of mineral contents and total base of the blood are made. Because citrate may interfere with the determination of uric acid or the precipitation of protein, and because it gives great disturbance in cell volume it should be avoided. If oxalate is neutral and if minimal amounts are used, no particular influence can be detected in the determination of the pH or carbon dioxide combining power. With but few exceptions, notably determination of calcium, sodium or potassium, oxalate may be used for all routine and even for special tests,

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* Since this paper was prepared, an exhaustive monograph dealing with anticoagulants, chiefly for chemical determinations, has been published by Blitstein.

† Heparin should not be used for the determination of phosphorus.

provided too much is not used. It has been found that if 1 cc. of 2 per cent solution of potassium or sodium oxalate is placed in a tube and dried, it will prevent clotting and will be satisfactory for from 5 to 20 cc. of blood. Such an amount will not interfere with the precipitation of protein or with most determinations for which whole blood is employed. According to Peters and Van Slyke, concentrations of oxalate of 0.3 per cent and more cause serious alterations in the constituents of the blood, and even frank hemolysis. Less concentration, when the oxalate is dry, as usually it is, causes changes in the volume of erythrocytes and hence determinations, for which plasma is employed, are subject to error.

Eisenman has pointed out that, if very accurate determinations of certain constituents of blood are necessary, oxalates are subject to question since they exert a varied and inconsistent influence on the electrolyte distribution in the blood. The errors, however, are of a small order and do not affect ordinary routine results. Eisenman did state that there was an absolute shrinkage of 14 to 15 per cent in the cell-volume when 0.4 to 0.6 per cent dry oxalate was used.

Several special anticoagulants have been advocated for special determinations, but for routine use, it has been found satisfactory to limit the anticoagulants to potassium or sodium oxalate and to make determinations on serum if methods in which whole blood is employed are unsuitable.

2. ANTICOAGULANTS FOR NONCHEMICAL TESTS OTHER THAN HEMATOCRIT DETERMINATIONS

There is a variety of tests, such as blood grouping, the determination of fragility, spectroscopic tests, the making of cell suspensions for biologic tests, and perhaps sedimentation tests, in which it is necessary to prevent coagulation and in which slight swelling or shrinking of the erythrocytes is not of importance.

A casual glance over texts and articles on the subject reveals a long list of anticoagulants which are recommended and various concentrations of them. For spectroscopic tests, it is often necessary to have water-clear solutions of laked blood, frequently in neu-

tral solutions, and hence it is best to place either the fresh blood directly in distilled water or, if certain dilutions are to be made, to defibrinate the blood and then lake it in whatever manner seems best.   For the other tests, except sedimentation tests, no particular discussion is needed.   Any of the citrates or oxalates is suitable and is best used in solution, and with just enough anticoagulant to prevent clotting.   However, it will be found advisable to perform fragility tests by dropping the fresh blood directly into various strengths of sodium chloride solution in a ratio of about one part blood to twenty or twenty-five parts of saline solution.

Numerous anticoagulants, and in various strengths, have been recommended for sedimentation tests.   Rourke and Plass, who maintained that inorganic anticoagulants depress the rate, based their recommendation for heparin on checks made with the blood of hemophiliacs.   Wintrobe and Landsberg did not support this contention.   It is evident that, if the blood is diluted with anticoagulants, some correction must be made against specimens in which dry anticoagulants are used.   Furthermore, it would appear reasonable, in the absence of statements to the contrary, that an anticoagulant that does not affect the volume of the erythrocytes should be used.   There is no unified opinion as to what anticoagulant or what amount should be used.   If an anticoagulant suitable for other tests can be used for sedimentation tests, there will be some advantage.

3. ANTICOAGULANTS FOR TESTS INVOLVING HEMATOCRIT DETERMINATIONS

It is here that critical work is imperative since the hematocrit determination is the basis for many calculations and of itself indicates important facts.   In view of this, it is interesting to note that other than for capillary tubes, the standard deviation of the test itself is not available.   The great number of anticoagulants and the variation in the amount advocated may be seen by examining table 1, which certainly does not list all that have been used.

Harvey, using 1.5 per cent sodium citrate in an 0.85 per cent

solution of sodium chloride, gave one protocol of six determinations on one specimen. From his figures, one may calculate that

TABLE 1

ANTICOAGULANTS USED IN HEMATOCRIT DETERMINATIONS

AUTHOR	YEAR	ANTICOAGULANT	AMOUNT OF ANTICOAGULANT	AMOUNT OF BLOOD
Deland...............	1891	2.5 per cent potassium dichromɛte*		
Capps................	1904	None		
Larrabee.............	1911	Dry sodium oxalate	1 part	100 parts
Keith, Rowntree, and Geraghty...........	1915	Dry sodium oxalate	q.s.	
Epstein..............	1916	Dry hirudin	q.s.	
Harvey...............	1919	1.5 per cent sodium citrate	1 part	1 part
Richter-Quittner......	1919	Hirudin	0.02–0.5 per cent	q.s.
Gram................	1921	3 per cent sodium citrate	0.5 cc.	4.5 cc.
Campbell.............	1922	Dry potassium oxalate	0.5 per cent	q.s.
Haden...............	1923	1.6 per cent sodium oxalate	2.0 cc.	10.0 cc.
Gram and Norgaard..	1923	Dry hirudin	ca. 1 mgm.	10.0 cc.
Osgood..............	1926	Dry sodium oxalate	20.0 mgm.	10.0 cc.
Jorgensen and Warburg...............	1927	None		
Rubin and Smith.....	1927	Dry hirudin	1.0 mgm.	5.0 cc.
Wintrobe and Miller..	1929	Dry potassium oxalate	40 mg.	10.0 cc.
Rowntree and Brown..	1929	1.6 per cent sodium oxalate	2.0 cc.	10.0 cc.
Haden...............	1930	1.4 per cent sodium oxalate	2.0 cc.	10.0 cc.
Murphy and Fitzhugh.	1930	1.6 per cent sodium oxalate	2.0 cc. (dried)	10.0 cc.
Rosahn..............	1931	Dry heparin	q.s.	
Graff and Clarke.....	1931	1.0 per cent potassium oxalate*	2.0 cc.	10.0 cc.
Wintrobe.............	1931	Dry potassium oxalate	10.0 mg.	5.0 cc.
Guest and Siler.......	1934	Dry heparin	0.5–2.0 mg.	1.0 cc.
Walters..............	1934	1.6 per cent sodium oxalate	1.0 cc.	5.0 cc.

* Cells examined microscopically.

the standard deviation was 1.07 per cent, although his series is of course small.

Norgaard and Gram compared defibrinated blood with blood mixed with 3 per cent sodium citrate, and concluded that hematocrit values checked if the citrate was used in the proportion of 1 to 9.

Campbell, from his experiments, concluded that his method of hematocrit determination gave an accurate reading to within 5 per cent, when capillary tubes and solid potassium oxalate were used.

Hooper, Smith, Belt, and Whipple made the first definite attempt to determine the strength of sodium oxalate which is isotonic with blood. They used the dog for the source of blood and decided on 1.6 per cent as the proper strength. In regard to the use of solid oxalate, they stated that 10 mgm. per 10 cc. of blood caused, on an average, a shrinkage of about 3 per cent in the cell-volume. The actual figures of their experiments are 55.1 per cent by the hematocrit for defibrinated blood, and 52.1 per cent for the oxalated specimen. This really means that there was a shrinkage of 5.4 per cent.* The use of 15 cc. centrifuge tubes caused certain error because of the wide meniscus in the tube, and hence the difficulty in reading; in addition, accurate readings were impossible because of the small linear space between markings. Further objections to these experiments were the failure to examine the cells microscopically for shrinkage (crenation) or swelling, the failure to examine the plasma spectroscopically for laking, and the failure to determine the standard deviation of the test. Furthermore, defibrination alters the blood considerably, and no evidence was presented to support the fact that addition of sodium oxalate after defibrination gives the same result as using it alone, and both methods are not used together in routine tests in determining an hematocrit value. The experiments were performed with the blood of the dog and there is no

* To give the proper significance to the term "per cent shrinkage" it is necessary to define the expression. It is the ratio of the difference between two hematocrit values to the greater value, or the ratio of the smaller to the larger hematocrit value subtracted from 100 per cent. It should not be confused, as it has sometimes been, with the difference in two hematocrit values expressed in terms of per cent (volume of erythrocytes per 100 cc. of blood).

evidence to prove that this blood and human blood are isotonic; hence, the use of 1.6 per cent sodium oxalate in observations on human blood is not justified from these experiments, although it is still used in certain laboratories.

Millar[12] stated that 10 mgm. of potassium oxalate to 10 cc. of blood had no appreciable effect on the diameter of the cells. While figures presented by Millar and others, in general, show very little difference in the diameter of the cells when various anticoagulants are used, hematocrit differences are significant and hence the volume of the cells is certainly altered.

Van Allen found that 0.1 per cent dry sodium oxalate produced a change in the volume of the corpuscle by 5.5 per cent. Using rabbits' blood from four experiments he concluded that 1.3 per cent sodium oxalate should be used for this type of blood. He pointed out that the work of Hooper and associates was open to question because of the fact that defibrination removes some erythrocytes along with the fibrin and that, as Descamps showed in peptone shock, the cells are reduced in size. These facts would tend to make the hematocrit values too small; hence, 1.6 per cent sodium oxalate would give only an apparent check. Four rabbits gave results which indicated that use of 1.28, 1.32 and 1.44 per cent of sodium oxalate checked with the "dry" hematocrit value. He cautioned against assuming that this strength would be suitable for human beings; nevertheless, he used it on the basis that the values in anemic blood would be comparatively accurate.

The small amount of blood used, the fact that each filling of the hematocrit tube had to be done from unpooled blood, and the fact that he gave no evidence by which one may judge the accuracy of the results or the possibility of repeating them, leaves some questions still to be settled. Using a "dry" hematocrit value as the final check is certainly open to criticism. Rabbits' blood clots rapidly and the centrifugal force employed was not great enough to assure packing before clotting. A feature of the Van Allen hematocrit is the small meniscus which allows more accurate reading than does a large tube but the small volume itself gives a source of error.

Osgood sought a single anticoagulant, which he could use for all kinds of determinations, and decided to use 20 mgm. of potassium oxalate per 10 cc. of blood. He averaged the results obtained with heparin, 3 per cent solution of sodium citrate and 1.6 per cent solution of sodium oxalate and compared the result with that obtained by use of dry potassium oxalate. He concluded the average shrinkage caused by the dry oxalate was 3.5 per cent. Evidently the tubes were not run in duplicate.

The experiments were altogether too few and the method of procedure was not well enough outlined to allow a definite conclusion to be drawn, but the results have been widely accepted and used.

Rowntree and Brown stated that 1.6 per cent solution of sodium oxalate on an average gave a volume of blood in the tube, which was 3.4 per cent higher than that obtained with the dry method.* This was based on a comparison of specimens from ninety-three subjects. "The range was from −1.6 to +9.6 per cent and the standard deviation of 2.4 per cent calculated as per cent of the volume of blood in the tube." In the only protocol recorded, a dog was given enough heparin to prevent its blood from clotting. Hematocrit determinations (five tubes) averaged 35.6 per cent. One cubic centimeter of 1.6 per cent sodium oxalate added to each of six tubes gave an average hematocrit value of 35.7 per cent. Sixteen milligrams of solid oxalate (six tubes) gave an average hematocrit value of 30.0 per cent. This is a difference of 5.7 per cent between dry sodium oxalate and heparin, and represents a shrinkage of 15.7 per cent. The standard deviations of these tests calculated from their recorded determinations are respectively 0.25 per cent, 0.50 per cent, and 1.0 per cent.

Wintrobe has several times reported on shrinkage in the hematocrit determination as a result of the use of solid oxalate. He and Miller tested 20 mgm. per 10 cc. of blood and reported shrinkage of 3.68 per cent, and shrinkage of 6.7 per cent when 40 mgm.

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* They stated that Whipple and his associates also found this value, but this is in error. In effect Rowntree and Brown added about 1.7 per cent to a value of 52 per cent, while Hooper (Whipple) and others would have added 3.0 per cent.

were used.*   In 1931, Wintrobe[22] stated that, on the basis of six determinations, he had revised the figure and that 20 mgm. in 10 cc. would cause a shrinkage on the average of 5.75 per cent in the volume of packed erythrocytes as compared to the volume of erythrocytes in heparinized blood.   In 1932 he[23] reported that 2 mgm. of potassium oxalate to 1 cc. of blood caused a shrinkage of 8.2 per cent (8.15 per cent in 1933[24]) in the volume of the erythrocytes, as compared with heparinized blood.   He gave, for a factor, 1.09.   Wintrobe and Landsberg reported that the anticoagulant of Heller and Paul† gave the same hematocrit reading as when heparin was used and advocated 6 mgm. of ammonium oxalate, 4 mgm. of potassium oxalate, and 5 cc. of blood.

Haden attempted to arrive at the proper anticoagulant by hirudinizing 100 cc. of blood from each of three subjects and then treating portions of it with various strengths of sodium oxalate solutions.   Averaging the results, it appeared that there was marked shrinkage with the saturated solution, that there was some increase with defibrinated blood, and that the control hematocrit values fell between those obtained with the 1.4 per cent and 1.6 per cent solution of sodium oxalate.   He repeated the general experiment with two other specimens of blood, using on different portions of each, 1.4, 1.5, and 1.6 per cent sodium oxalate, as well as more concentrated solutions, and dry oxalate.   Again, there was marked shrinkage (8.25 per cent with 20 mgm. per 10 cc.) with the higher concentrations.   Haden, although he had pointed out that equilibrium was not reached until after at least an hour's centrifuging, averaged the half-hour, one-hour, and two-hour values.   While obviously not mathematically correct, his conclusions would not be affected by a more correct procedure.

* Wintrobe has apparently used the same value either to bring a higher figure into conformity with a lower one or vice versa.   In addition, Wintrobe and Miller, instead of calculating the shrinkage, calculated the percentage that the hematocrit reading of heparinized blood was greater than that with oxalated blood.   For example, their figure of 6.98 per cent should have been 6.5 per cent to have been placed on the same basis as Osgood's.

† They compared this anticoagulant with dry oxalate and citrate, but not in determinations on human blood.

He concluded that 1.4 per cent sodium oxalate acted more like hirudin than sodium oxalate of any other percentage, although his results were not in exact accord with those of the first experiment and hirudin had first been added to the blood which was later oxalated.   He recorded ten determinations of hematocrit tests, using heparin and 1.4 per cent sodium oxalate independently. The average with heparin was 44.95 per cent, and with oxalate it was 44.82 per cent.   Apparently, no tubes were run in duplicate, and since he did not provide a standard deviation, there is no way of deciding what values were significant.   He evidently did not examine the corpuscles under the microscope to note swelling or crenation.

Rosahn, using heparinized rabbits' blood in capillary hematocrit tubes, determined the accuracy of the method on ten animals, using ten tubes for each.   His standard deviation ranged from 0.2 to 0.8 per cent, with an average of 0.5 per cent.

Foster and Johnson found that 8.5 per cent of the observed reading must be added if oxalated blood were used (20 mgm. per 10 cc.) to make it equal the observed reading with heparinized blood.   Using 1.3 per cent sodium oxalate, the average hematocrit value for forty human beings was 44.4 per cent, while with the heparinized blood it was 46.7 per cent, a difference of 2.3 per cent in volume, or a shrinkage of 4.9 per cent.

Graff and Clarke are apparently the only authors who have applied the method of examining the corpuscles to determine the morphologic effect of oxalate used as an anticoagulant.   The results were read after five hours and after twenty-four hours, and they used heparin to prevent clogging of the oiled tubes used in the experiments.   They carried out fourteen experiments on human blood under suitable precautions and in none was hemolysis observed with solutions containing 1 per cent or more of potassium oxalate.   Hemolysis was noted in some specimens in lower concentrations.   Crenation occurred in every specimen containing 1.1 per cent or more, and in half of those which contained 1.0 per cent of potassium oxalate.   They adopted the 1 per cent solution as optimal and used 2 cc. to 10 cc. of blood.   According to Ponder and Saslow, crenation may not necessarily be

associated with decreased volume, since increased volumes are sometimes observed when the cells are crenated.

Guest and Siler studied the effect of various anticoagulants. The tests were done with capillary hematocrit tubes. One sample of blood was divided into two portions, one of which was treated with 0.5 mgm. of heparin per cubic centimeter and the other with 2.0 mgm. per cubic centimeter. From each of these portions eight pairs of tubes were filled. The average hematocrit values determined on both groups of tubes were without significant difference: $41.92 \pm .11$ and $41.84 \pm .11$. It is significant to note the fact that defibrinated blood gave a smaller hematocrit value than that obtained when dry heparin or hirudin were used, and that sodium oxalate (dry) gave an hematocrit value 5.6 per cent lower than did heparin; hence, a shrinkage of 12.4 per cent. Readings were made with a special measuring microscope.

EXPERIMENTS

All experiments were carried out with human blood in the Sanford-Magath hematocrit tube. This is graduated to 6 cc. and has an over-all length of about 9.5 cm. The diameter is small, hence the meniscus can be read with accuracy, and the linear distance between 0.1 cc. markings is great enough to allow interpolations to 0.02 cc. with relative accuracy. As a rule, 5 cc. of blood was used to 1 cc. of anticoagulant. The tubes were calibrated with water at room temperature. Centrifuging was done at 3000 revolutions per minute, with an arm radius of 14 cm. Packing was constant within one hour; hence, all tubes were centrifuged for one hour after being sealed with rubber caps. The importance of these caps cannot be overemphasized, since in an hour's centrifuging, evaporation of more than 0.5 cc. may take place. Although calculations for complete packing were made by a modification of Hirota's formula, these will be reported later, together with a discussion of his theory. The values given here were not so calculated. Heparin was secured from the Connaught Laboratories and was 5 unit strength. The mixtures were examined microscopically for changes in the cells, and the plasma was tested spectroscopically for hemolysis.

From an examination of twenty-five specimens of human blood after they had been allowed to remain in contact with various strengths of solutions of sodium oxalate for two hours, it became apparent that crenation developed when the concentration was greater than 1.1 per cent and increased in severity as the concentration increased. The greatest crenation was observed when dry oxalate was used. Swelling became apparent as the concentration decreased below 1.1 per cent and hemolysis was observed in concentrations below 0.8 per cent and occasionally in concen-

TABLE 2

The Standard Deviation of the Hematocrit (Single Tube)

Five sets of observations of ten tubes each from five subjects

(Anticoagulant, 1 cc. of 1.1 per cent sodium oxalate; blood, 5 cc. centrifuged to a constant volume.)

EXPERIMENT	AVERAGE OF HEMATOCRIT VALUES	SIGMA
	per cent	*per cent*
1	42.95	0.30
2	47.05	0.49
3	48.30	0.75
4	50.31	0.72
5	51.21	0.68
Average..		0.60*

* The average value of the standard deviation is 0.60 per cent. This means that there is a two-thirds chance that a single determination is correct within ± 0.60 per cent, and that a single observation is significantly determined within 1.20 per cent (twice the standard deviation).

trations as high as 1.6 per cent. From these experiments, it was decided to test 1.1 per cent sodium oxalate thoroughly.

In table 2 is recorded the standard deviation of the test, which is 0.60 per cent. Therefore, any value determined with a single tube is significantly determined within 1.20 per cent with this method. This checks with the results reported by Guest and Siler, and by Rosahn, and shows that this method is as accurate as theirs, probably because of the larger amount of blood used by us. From the standard deviation given, one may calculate the standard deviations for multiple tubes of the same blood by divid-

ing 0.60 per cent by the square root of the number of tubes used; thus, if a pair of tubes are averaged, the standard deviation is 0.42 per cent $(0.60/\sqrt{2})$; for ten tubes, it is only 0.19 per cent $(0.6/\sqrt{10})$. This standard deviation includes all the errors of the test, including such items as variations in the ability to read the level of the meniscus, variations in packing at constant centrifugal force, and other physical and personal factors such as exist in all biologic tests. It is known that carbon dioxide equilibrium also affects the volume of the erythrocytes, but all tubes were immediately stoppered and centrifuged promptly with rubber caps; whatever error is caused by this factor is also included in the calculated standard deviation.

Since there is abundant evidence in literature to justify the conclusion that heparin may be used as an anticoagulant without affecting the size and shape of the erythrocytes, heparin was used as a standard in the proportion of 2 mgm. per 5 cc. of blood. One must exert the greatest care when using dry heparin in order to bring it into solution in the plasma and thus to avoid minute clots.

The values obtained for ten individuals were compared with those obtained with dry sodium oxalate in the proportion of 2.2 mgm. per 1 cc. of blood, since this conformed to amounts used by previous investigators. From table 3, it may be seen that there is a great deal of shrinkage, an average of 11.30 per cent, and that to translate such values to readings with heparin, it is necessary to multiply by a factor of 1.127. Furthermore, the readings of duplicates were not so close together as they were when liquid anticoagulants were used.

In order to establish the hematocrit value for concentrations of sodium oxalate of about 1.1 per cent, a series of hematocrit values based on the use of 0.9, 1.1, and 1.3 per cent sodium oxalate was compared with values based on the use of heparin. It can be seen from table 4 that 1.1 per cent sodium oxalate may be considered entirely satisfactory and that results when it is used check very accurately with results obtained when heparin is used. Another set of ten determinations checked these values. Changing the ratio from a half to four times the amount of solution of

1.1 per cent sodium oxalate, to the volume of whole blood, does not produce any significant change in the hematocrit value, which is also true in the case of 1.3 per cent sodium oxalate solution.

A few experiments were tried, using potassium oxalate in place of sodium oxalate, and while enough tests were not done to confirm the results completely, it appeared that 1.0 per cent potas-

TABLE 3

COMPARISON OF HEMATOCRIT DETERMINATIONS WHEN WET HEPARIN AND DRY
SODIUM OXALATE ARE USED

(Each value represents an average of two determinations)

EXPERIMENT	2 MG.* HEPARIN	11.0 MG.* DRY SODIUM OXALATE
	per cent	per cent
1	47.35	42.75
2	50.56	45.33
3	47.47	42.45
4	43.99	38.77
5	42.37	36.81
6	45.28	40.49
7	43.88	40.49
8	43.66	37.48
9	42.45	36.50
10	48.85	43.10
Average..............	45.58	40.42

* Per 5 cc. blood.

There is an average difference of 5.16 per cent in hematocrit values when dry oxalate is used. This value is determined significantly within $\pm$ 0.19 per cent $(0.6/\sqrt{10})$. The shrinkage amounts to 11.30 per cent of the volume obtained by the use of heparin. One may, therefore, translate a reading made with dry oxalate to its heparin equivalent by multiplying by 1.127 (12.7 per cent).

sium oxalate gave results exactly equivalent to 1.1 per cent sodium oxalate. A few experiments were tried, using the anticoagulant (ammonium oxalate 40 per cent and potassium oxalate 60 per cent) recommended by Heller and Paul. There was statistically significant shrinkage as compared to that when 1.1 per cent sodium oxalate was used.

Plotting the effect of various concentrations of sodium oxalate indicates that a large amount of swelling takes place as the con-

centrations are decreased to less than 1.1 per cent, while increasing the concentration to more than 1.1 per cent produces the greatest shrinkage between 1.1 and 1.3 per cent; the effect of increasing the concentration between 1.3 and 1.6 per cent is not so great.   This is shown in figure 1.

TABLE 4

COMPARISON OF HEMATOCRIT VALUES, USING VARIOUS STRENGTHS OF SODIUM OXALATE

(1 cc. of anticoagulant and 5 cc. blood; each value represents an average of two determinations)

EXPERIMENT	2 MG.* HEPARIN	SODIUM OXALATE		
		0.9 per cent	1.1 per cent	1.3 per cent
	per cent	*per cent*	*per cent*	*per cent*
1	54.79	57.09	54.49	54.03
2	49.61	52.04	50.73	48.47
3	47.18	48.18	47.07	46.69
4	49.66	49.35	49.95	48.60
5	42.32	42.51	42.68	41.21
6	50.32	51.35	50.81	49.54
7	49.50	50.20	49.49	47.99
8	43.73	44.72	43.98	42.90
9	46.17	47.52	46.26	45.27
10	48.22	49.70	48.25	47.20
Average......	48.15	51.27	48.38	47.19

* Per 5 cc. blood.

Each determination is an average of two observations; therefore, the standard deviation of these determinations is 0.42 per cent $(0.6/\sqrt{2})$. The standard deviation of the difference between any pair of different anticoagulants is 0.6 per cent $(\sqrt{2} \times 0.42)$ and such a difference must be at least 1.2 per cent $(2 \times 0.6$ per cent) to be considered significant.   Since the final results are based on an average of ten, the errors are divided by $\sqrt{10}$ and a difference in these averages of 0.38 per cent is significant.   There is no significant difference between results obtained with heparin and with 1.1 per cent oxalate, but the difference between results obtained with heparin and with 0.9 per cent oxalate, as well as with 1.3 per cent oxalate, is significant.

All of these experiments just recorded were performed in such a manner that the tubes were centrifuged promptly after taking the blood and in no case did the tubes stand for longer than two hours before the test was completed.   When blood was mixed with
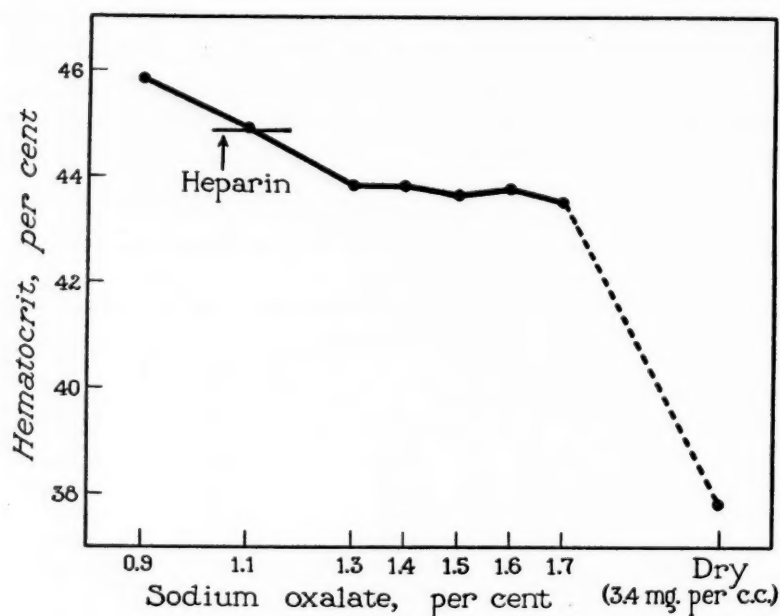
FIG. 1. The Effect of Various Concentrations of Sodium Oxalate on
the Immediate Hematocrit Value

TABLE 5

Effect of Time on Hematocrit Values

Averages of ten experiments

HEPARIN	SODIUM OXALATE							
	1.1 per cent				1.6 per cent			
	No standing	After 2 hours	After 4 hours	After 6 hours	No standing	After 2 hours	After 4 hours	After 6 hours
per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
47.45	47.74	48.41	48.74	48.98	46.06	46.46	46.54	47.03

The standard deviation of the difference between the value with heparin and
that of any other value is 0.265 per cent, and such a difference must be at least
0.53 per cent to be considered significant. There is no significant difference be-
tween values obtained with heparin and those obtained with 1.1 per cent sodium
oxalate up to two hours of standing, or between those obtained with heparin and
those obtained with 1.6 per cent sodium oxalate after standing six hours. The
factors by which intermediate hematocrit values may be corrected to equal
values obtained with heparin are as follows: for 1.1 per cent sodium oxalate, two
hours, 0.980; four hours, 0.973; and six hours, 0.969; for 1.6 per cent sodium oxalate,
under two hours, 1.030; two hours, 1.021; and four hours, 1.019.

oxalate solution and allowed to stand, a very different effect was produced.   When 1.1 per cent sodium oxalate was used as an anticoagulant, no significant change in the hematocrit value was observed until after the mixture of blood and oxalate had stood about two hours.   Thereafter, there was a small though significant rise in the hematocrit value.   It was also observed that when 1.6 per cent sodium oxalate was studied from this standpoint there was an immediate shrinkage in the hematocrit value which was gradually restored on standing six hours until there was no significant difference between such values and those obtained with heparin.   Table 5 indicates these changes.

<div align="center">COMMENT</div>

Hematocrit determinations, which were first described by Hedin, were made on unaltered blood with the Blix instrument. It was soon seen that it was necessary to have the patient or animal close to the centrifuge and that great speed and centrifugal force were necessary to pack the erythrocytes before clotting took place.   Since the actual error of such a method has not been determined and since the method was not so very practical, it has been abandoned by most investigators in favor of a method which prevents coagulation.   While heparin is perhaps an ideal anticoagulant, it has the disadvantage of being expensive and not particularly easy to handle.   Since equally accurate results can be obtained with 1.1 per cent sodium oxalate under the conditions indicated, this will be found applicable in the routine clinical laboratory.   It is of use in practically every test in which it is desired to prevent coagulation.   Any one of the commonly employed sedimentation tests may be executed, with suitable modifications, by using this as the anticoagulant, and since it will not produce significant changes in the volume of the cells for several hours, it may even be more suitable than other anticoagulants.

While 1.1 per cent sodium oxalate is not exactly isotonic for human blood, about 100 different specimens have failed to show changes detectable with the microscope or spectroscope on standing two hours.

If one performs the hematocrit determination as outlined in

these experiments, the standard deviation may be expected to be of the order of 0.60 per cent, and for any use so far proposed for the hematocrit, this may be considered entirely satisfactory. It is likely that, in view of the results herein reported, it will be necessary to recalculate some of the results previously published by others in regard to blood volume, size of cells, cell volume, and volume index. It is evident, not only from results that already have been published, but also from those obtained in this study, that considerably more shrinkage is caused by dry oxalate than commonly is supposed, and its use, even in counting the corpuscles and in the determination of hemoglobin, should be carefully restudied.

Comparing hematocrit values obtained with various oxalate solutions with those obtained with crude heparin or with defibrinated blood, and with oxalated blood previously heparinized or hirudinized probably has resulted in the different values obtained by other authors, and the few specimens studied without a knowledge of the error of their methods also have resulted in different interpretations. It is also evident that differences in results have been obtained because of the use of different intervals of time between the mixing of the anticoagulant with the blood and the beginning of the process of centrifuging.

It is unknown just what physical effects take place when sodium oxalate comes in contact with blood. Tests kindly performed for us by Dr. E. J. Baldes indicate that when blood and 1.6 per cent sodium oxalate are mixed the plasma has the same tonic value as 0.92 per cent sodium chloride or heparinized blood plasma, while 1.1 per cent sodium oxalate and blood give values slightly lower. Yet the hematocrit values obtained immediately after the mixing are more nearly like those obtained by the use of heparin when 1.1 per cent sodium oxalate is used than when 1.6 per cent sodium oxalate is used. It would appear that when oxalate stronger than 1.1 per cent is used the effect is to withdraw water immediately from the cells and that this is then slowly taken up by the cells, requiring about six hours before the volume is completely replaced if 1.6 per cent oxalate is used. On the other hand, when a 1.1 per cent solution of oxalate is used, the

cell does not begin to take in water until a lag period of two or three hours occurs; then it slowly absorbs water for six or eight hours. Hence, the time factor is important as well as the factor of concentration of oxalate.

Many factors enter into the hematocrit determination and our experiments dealt specifically only with the matter of the strength of the anticoagulant and time. Millar[13] has pointed out the variation caused by the centrifugal force exerted, which is in turn a function of time, speed, type of tube used, and length of radius of the head of the centrifuge. We were concerned with setting up a practical method which is simple. This may be easily repeated and gives a result accurate enough for practical purposes. We have established the degree of error one may reasonably expect and our experiments seem to clarify to some extent the situation in regard to the anticoagulant. Hematocrit tests on unaltered blood are certainly unreliable, on the whole, since partial coagulation occurs and the volume usually is, therefore, too great, according to Ponder and Saslow.

CONCLUSIONS

(1) The standard deviation of the hematocrit test as performed with the Sanford-Magath tube is 0.60 per cent.

(2) Heparin produces no swelling, crenation, or laking.

(3) Dry oxalate produces a great deal of shrinkage of erythrocytes. When 22 mgm. of dry oxalate per 10 cc. of human blood is used, the average hematocrit value is 5.16 per cent less than that obtained with wet heparin, which indicates a shrinkage of 11.30 per cent. It is necessary to multiply the value for dry oxalate by 1.127 to equal the true hematocrit.

(4) Sodium oxalate in 1.1 per cent solution gives an hematocrit value equal to that obtained with heparin and does not cause human erythrocytes to swell or become crenated nor does human blood become laked, as observed microscopically and spectroscopically, provided the centrifuging is done before two hours have elapsed. For practical purposes, this anticoagulant may be considered suitable for human blood. If the mixtures of oxalate and blood are allowed to stand the following factors must

be used by which to multiply the per cent of erythrocytes: after
two hours, 0.980; after four hours, 0.973; and after six hours, 0.969.
If 1.6 per cent sodium oxalate be used, one must use the following
factors: less than two hours, 1.030; two hours, 1.021; and four
hours, 1.019; at six hours, the value is not significantly different
from values obtained with heparin.

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EDITORIAL

The Selection of Antiseptics and Disinfectants

The search for ideal antiseptics and disinfectants, that is those which will inhibit or destroy bacteria and still leave the tissue cells individually and collectively unharmed, is a medical problem dating almost from the discovery of pathogenic bacteria. It goes almost without saying that the ideal has not been discovered, but many different substances have been tested and found effective in varying degrees against certain groups of bacteria in particular systems or tissues of the body. In analyzing the situation there appear to be two principal reasons for not having more nearly approached the ideal, first inadequate methods of testing, evaluating and comparing antiseptics and disinfectants, second because the scientific assay of these substances such as they are have been disregarded by the profession through misinformation and indifference, disregarded by manufacturers through prejudice and competition and disregarded by the public through lack of knowledge and through exploitation on the part of distributors and manufacturers.

Regarding methods of examination, Reddish pointed out that the phenol coefficient test was the natural outgrowth of the early use and constant properties of phenol. The standard phenol coefficient tests were based on the use of *Eberthella typhi* but as both Tilley and Reddish have pointed out an antiseptic that is effective against typhoid bacilli may be weak or ineffective against *Staphylococcus aureus* and other organisms. For example, certain chloro-phenol compounds show reported coefficients as low as 20+ against *Eberthella typhi* and 1200+ against *Staphylococcus aureus*. Such striking differences in effectiveness are more apt to be noted with simple substances than with compound substances or mixtures. Merthiolate as an instance has a phenol coefficient of 50 using *Eberthella typhi* and 70 using *Staphylococcus*

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aureus. Not only are compounds and mixtures more uniform in their activity but the activity is also enhanced beyond predicted levels as noted in the author's work with phenols, thymols, resorcinols and tricresols used in combination with certain organic acids as citric, tartaric, benzoic, salicylic and tannic acids and inorganic acids especially hydrochloric. Such combinations of relatively inactive and non-toxic substances give phenol coefficients five to fifteen times the combined coefficients of the two substances when used alone and bring their activity well within the range of the mercury compounds and mixtures without increasing their toxicity.

To increase the specific information obtainable from phenol coefficients Reddish recommended the use of various pathogenic organisms to be used in addition to *Eberthella typhi* and outlined methods for their use. Hence one must know the method and organisms used in doing the phenol coefficients as well as the results of the test just as one must know the type of serological test used for the diagnosis of syphilis as well as the result.

These improvements introduced by Reddish along with tests for penetration, as the agar cup method of Allen, give adequate methods for evaluation and comparison of the activity of antiseptics both as regards the type of organism and conditions encountered in clinical practice. The other large factor to be evaluated and compared is general and local toxicity. General toxicity is readily determined by intraperitoneal and intravenous injection of the antiseptic. Local toxicity was studied by Lambert, Lambert and Meyer, German, Buschsbaum and Bloom and most recently by Salle and Lazarus, utilizing tissue cultures. The last named investigators evolved the following formula:

$$\text{"toxicity index"} = \frac{\text{highest dilution showing no tissue growth}}{\text{highest dilution showing no bacterial growth}}$$

These methods for determining local toxicity have not been standardized as yet but promise much in the evaluation and comparison of antiseptics from the standpoint of tissue cell survival and wound healing. It is quite probable that various types of

tissue cells will have to be used in the ultimate development and standardization of these tests.

After careful laboratory evaluation of antiseptics and disinfectants through a study of phenol coefficients and toxicity indices it remains for the clinician to select the most effective material for the particular invading organism or group of organisms as determined by culture. Data regarding the various antiseptics and their effect on particular organisms along with toxicity indices is available in the current medical literature, but this is apparently overshadowed by advertisements, manufacturers propaganda and high pressure salesmanship to such an extent that the most widely heralded material becomes the one used for all purposes from the treatment of minor wounds to skin sterilization, and from treatment of large burns and infected cavities to intravenous injection for septicemia regardless of the infecting organism or the local and general toxicity.

With our rapidly increasing group of active antiseptics and our increasing efficiency in their evaluation the profession should be more critical in their selection and more discriminating in their application approaching the ultimate goal of specific therapy.

F. W. Hartman.

NEWS AND NOTICES

Abstract of the Minutes of the 1935 Meeting of the American Society of Clinical Pathologists in Atlantic City, New Jersey*

The business meeting was called to order by President Lamb at 8:30 P.M. on June 9, 1935. Seventy-five members were present. The minutes of the previous meeting were approved as published in the official JOURNAL.

REPORT OF THE SECRETARY-TREASURER

The total membership of the Society as of June 1935 was 405, 365 members have paid their dues, thirty-one are in arrears for one year, nine for two years, and twenty-four were dropped from the roster for non-payment of dues. Five members resigned and one died.

The financial report, presented in detail to the Executive Committee, reveals for the year of 1934 a total income of $5,142.75 and total expenses of $3,976.78, bringing the net-worth of the Society to $6,643.32. The treasurer is glad to report that the balance held in the bank at the time of the bank moratorium has so far been paid to the extent of eighty-five percent of the deposits.

The report was accepted as read.

EXECUTIVE COMMITTEE REPORT

Your Committee examined the accounts and books and certified public accountant's report of the Secretary-Treasurer and found them to be in perfect order. We commend him on the excellent and economical administration of his office.

Because of the rapidly increasing volume of work necessarily handled by his office we recommend an increase in the salary allottment of his budget.

The Registry of Technicians report is heartily approved with deep gratitude to the Committee for the large amount of work entailed in their efforts.

The affairs of the JOURNAL are particularly gratifying to the Committee of which we are justly proud. We compliment Dr. Magath, the Editor, on his

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* Due to the length of many of the reports, it is found necessary to abridge many of them and publish only the salient points.

splendid efforts. We recommend the appropriation of $500.00 as in previous years, for expenses of his office.

We recommend to the Society the endorsement of the report of the Committee on Qualifications.

In view of the intense interest shown by the Society in the matter of hematology we endorse the suggestion that a day be allotted to a Hematological Conference in next year's preconvention meeting.

We recommend that the report of the Public Relations Committee be submitted to the Committee appointed for 1935–36 with the request that this Committee submit to the Executive Committee a plan of action based upon these recommendations and such other suggestions as the new Committee may add within 60 days from their appointment.

We endorse the resolutions to be presented concerning the matter of the publication of a book on laboratory methods.

In view of the fact that records of the A.S.C.P. reveal that Dr. J. A. Kolmer and the associated editors were given complete authority to arrange all details for the publication of a book on approved laboratory methods, and in view of the fact that the matter of distribution of royalties accruing from the sale of the book was to be left with the Editor, Associate Editors, and assisting committee, it is moved that the executive committee reaffirm these previous actions and leave the matter of distributions of the accrued royalties from the sale of the first Edition to the judgment of Dr. Kolmer as per the understanding arrived at in 1928.

Moved by Dr. Simpson and seconded by Dr. Owen. Carried unanimously.

A resolution was presented by the Executive Committee, that a new edition of the book entitled Approved Laboratory Technique be authorized under the editorship of Doctor John A. Kolmer with suggestions of new features to be introduced into the book.

The resolution was approved as read.

REPORT OF THE JOURNAL

The JOURNAL has had a most successful year and the Editor is pleased to report a profit, due to the sale of the monograph and sale of the JOURNAL, of $273.16. This is after paying for an increase of ten per cent in the number of pages in the JOURNAL. The number of subscriptions and the advertising to the JOURNAL have materially increased and it is expected that the following year will show an even larger profit. It is the intention to increase the size of the JOURNAL with the profits received. The JOURNAL is abstracted in practically every abstracting journal and the numerous references to it indicate that it is being widely read.

REPORT OF THE COMMITTEE ON QUALIFICATION OF PATHOLOGISTS

Before presenting the final report Dr. Sanford spoke extemporaneously in reference to the problem in general in connection with the formation of a qualifying board for Pathologists, and he pointed out many reasons for the establishment of such a board.

The Committee appointed to consider the possibilities of forming a Qualifying Board for Pathologists in conformity with the desires of the Advisory Board on Medical Specialities met at a meeting in Atlantic City, June 7th, 1935.

They are agreed that such a Qualifying Board should be formed at this time, and resolved that a Committee of three or more be appointed to meet with a similar committee from the Section of Physiology and Pathology of the American Medical Association with power to act with the approval of the Executive Committee in the immediate formation of a Specialty Board in Pathology.

Report accepted.

REPORT OF THE PUBLIC RELATIONS COMMITTEE

The report was read and a motion made by Dr. Exton that it be received and filed as recommended by the Executive Committee and that this report be referred to the next Public Relations Committee for action. Dr. Lamb pointed out that the Executive Committee has spent considerable time in studying this report and recommended that it be submitted to the incoming Public Relations Committee for recommendations within sixty days from the date of the meeting.

REPORT OF THE NECROLOGY COMMITTEE

Dr. F. C. Payne presented the report of the Necrology Committee and presented the following resolutions:

Be it resolved, therefore, that we the members of the American Society of Clinical Pathologists do mourn and regret the untimely death of our fellow member, Dr. Warren Buxton Stone.

Be it further resolved, that these resolutions be spread upon the minutes of this Society and that a copy be sent to his bereaved.

Motion made and carried for acceptance.

REPORT OF THE NECROPSY COMMITTEE

Dr. Davidsohn presented the report of the Necropsy Committee. The committee during the last year has engaged in two projects. One is the compilation of a bibliography on the subject of necropsies for the years 1931–1934. This material is available to any member of the Society who may need it for the preparation of articles on the subject and may be obtained at the Secretary's office. It is the plan of the committee to continue this work in the future.

The second project was the compilation of information on the number of instances in which the relatives obtained any material information of value directly or indirectly to them from a post mortem examination. The purpose of this data, obviously, is to help in obtaining permissions for autopsies.

Report was accepted as read.

REPORT OF THE BOARD OF REGISTRY

The Board of Registry reports a remarkable growth in the number of applicants for registration due to the recommendations by the A. M. A. and the American College of Surgeons, that approved Clinical Laboratories should have registered technicians.

The Board of Registry is fully appreciative of the excellent cooperation given them by Fellows who participated in examining the tremendously large number of applicants.

During the fiscal year, 287 names were added to the roll as Laboratory Technicians and 9 as Medical Technologists. The total registration of Laboratory Technicians is 2320 and Medical Technologists is 101.

Due to the cooperating efforts of the A. M. A. and the Registry the weaker training schools for technicians are gradually being eliminated.

The Registry submitted a financial report, compiled by a Certified Public Accountant, and the report was approved by the Executive Committee.

The report was approved as read.

REPORT OF THE RESEARCH COMMITTEE

Dr. Kline commented on the activities of the Serologic Division and referred to the Serologic Conference as part of the accomplishments of the projects carried out.

The following resolution was presented:

Whereas, the Committee on Serologic Conference of the A. S. C. P., composed of Arthur H. Sanford, Walter M. Simpson, and A. S. Giordano, has fulfilled its duties to the Society, and

Whereas, the project for the evaluation of serodiagnosis of syphilis in the United States has been completed, and

Whereas, the A. S. C. P. is fully cognizant of the great importance of the cooperation of the United States Public Health Service in conducting this study,

Be It Resolved, that the A. S. C. P. does hereby extend this expression of its deep gratitude to Doctor Hugh S. Cumming, Surgeon General, U. S. Public Health Service, to Doctor John McMullen, Assistant Surgeon General and Chief of the Division of Venereal Diseases, United States Public Health Service, to Doctor R. A. Vonderlehr, Past Assistant Surgeon, United States Public Health Service, to Doctor George W. McCoy, Medical Director, United States

Public Health Service, to Doctor H. H. Hazen and Doctor Francis E. Senear, Special Consultants, United States Public Health Service, and to the participating serologists (Doctors Brem, Eagle, Hinton, Johns, Kahn, Kline, Kolmer, Kurtz, Lufkin, Rytz, Rein, Ruediger, Williams and Weiss) for their diligent and unstinted efforts in bringing this project to a successful conclusion, and

Be It Further Resolved, that copies of this Resolution be sent by the Secretary to Doctor Cumming, Doctor McMullen, Doctor Vonderlehr, Doctor McCoy, Doctor Hazen, Doctor Senear, and to each of the participating serologists.

Unanimously approved.

Dr. Brines reported on the Tumor Registry and pointed out that the committee was discouraged by the lack of response from the general membership in submitting material to study. The committee hopes that in the future better cooperation will be given to the committee in carrying out the work.

The report was accepted as read.

Dr. R. R. Kracke, chairman of the Hematological Division, reported that to date 136 cases of unusual blood dyscrasias with complete records are on file in the Registry. Nearly all of these include autopsy material.

It was emphasized that the Registry performs two functions. First, that of collecting a large amount of valuable material for study and second, acting as a consultant to those submitting cases.

This year the committee is planning to collect a number of representative samples of the more common blood disorders so that they may be available to the membership for study.

The report was accepted as read.

Dr. Johns reported for the Committee on Fungi. The committee reported that during the two year period that this committee has existed it has been found impractical to establish a bureau for the identification of molds. The committee has surveyed medical literature for the last thirty years and has available a bibliography containing 7000 references. It is hoped that this bibliography may be added on to and in the near future be published. A summary of the preliminary studies on the possibility of identifying pathogenic fungi on material obtained from lesions together with confirmatory cultural requirements will be presented for publication by the Committee shortly.

The report was accepted as read.

REPORT OF THE LIFE MEMBERSHIP COMMITTEE

Dr. Hunter reported that after consideration and investigation of a number of different plans, now in operation in other societies, that it is quite desirable

that the A. S. C. P. adopt a plan of Life Membership and Bequests, and the committee submitted the following recommendations:

(1) That life membership be established in the American Society of Clinical Pathologists, and that the same be eligible to active members and associate members.

That an active and associate member be accorded life membership up to 45 years of age by the payment of $200 at the time of election to stated membership.

From 45 years of age to 58 years of age, the member shall pay an amount equivalent to the total sum of dues he would ordinarily pay from his present age to the age of 65.

From the age of 58, upward, the minimum life membership fee shall be $100. For example, up to and including age 45, $200; age 46—19 years—$190; age 50—15 years—$150; age 55—10 years—$100; and 59 and thereafter, $100 minimum.

(2) It is further recommended that a Bequest Fund be established by the Society, and that a committee shall be appointed on the gaining of bequests, and that the following objects be outlined:

To establish a fund for defraying the expenses of publication of the transactions of the Society.

To provide for funds for indigent members of the Society who are in need of hospitalization or lack the necessities of life.

To establish a fund for investment, the interest of which would be used for medical research of members as may be approved by the Executive Committee.

For the promotion of the field of clinical pathology and for any other purpose that may be desired.

(3) That cash bequests be placed in a separate fund, legacy fund, that the principal be wisely invested by the Secretary-Treasurer with the particular object of maintaining the funds intact and safe-guarding the principal; that the income only should be used in the discretion of the Executive Committee in carrying out the wishes expressed by the benefactors. Bequests for special purposes placed in this fund, should be designated and accounted for separately.

Report accepted.

REPORT OF THE BOARD OF CENSORS

The report by Dr. Maynard submitted 39 new members who were accepted and the names were published in the July issue of the Journal.

CHANGE IN THE CONSTITUTION

The following change in the Constitution was passed:
Article III—Section 2.

... They shall be members in good standing of their county and state and/or provincial medical society and of the American Medical Association or the Canadian Medical Association. ...

REPORT OF THE NOMINATING COMMITTEE

The report of the nominating committee on the election of officers was published in the July issue of the JOURNAL.

A motion was made by Dr. Foord expressing the gratitude of the Society to Dr. Kilduffe and his associates for the splendid preparations made for the annual meeting. The motion was unanimously adopted.

Meeting adjourned.

LIST OF APPOINTED COMMITTEES FOR 1935–1936

Committee on Commercial Exhibits
Dr. A. W. Freshman, *Chairman*
Dr. Fred Boerner
Dr. A. V. St. George
Dr. Herbert R. Brown
Dr. A. S. Giordano
Dr. Robt. Koritschoner

Committee on Code of Ethics
Dr. C. I. Owen, *Chairman*
Dr. C. M. Hyland
Dr. A. S. Giordano

Committee on Certification of Pathologists
Dr. A. H. Sanford, *Chairman*
Dr. J. H. Black
Dr. A. H. Braden
Dr. F. E. Sondern

Committee on Life Membership
Dr. O. B. Hunter
Dr. R. C. Beck

Necrology Committee
Dr. G. B. Kramer, *Chairman*
Dr. T. C. Terrell
Dr. F. B. Johnson

Committee on Necropsies
Dr. I. Davidsohn, *Chairman*
Dr. C. A. Hellwig
Dr. O. Saphir
Dr. Margaret Warwick

Committee on Public Relations
Dr. L. W. Larson, *Chairman*
Dr. C. W. Maynard
Dr. C. I. Owen
Dr. A. G. Foord

Program Committee
Dr. A. S. Giordano, *Chairman*
Dr. F. J. Heck
Dr. S. P. Reimann

Publicity Committee
Dr. T. B. Magath, *Chairman*
Dr. A. S. Giordano
Dr. Norbert Enzer
Dr. M. P. Neal

Committee on Round Table Program
Dr. R. R. Kracke, *Chairman*
Dr. Russell Haden
Dr. Frank Heck

Committee on Seminars
Dr. Fred Lamb, *Chairman*
Dr. Walter Simpson
Dr. A. S. Giordano
Dr. J. J. Moore

General Research Committee
Dr. R. R. Kracke, *Chairman*
Dr. H. M. Banks
Dr. Anna Mae Young

Hormone Division

    Dr. Anna Mae Young, *Chairman*

    Dr. Harry L. Reinhart

    Dr. E. R. Mugrage

Tumor Registry

    Dr. O. A. Brines, *Chairman*

    Dr. S. P. Reimann

    Dr. J. C. Bugher

    Dr. Everett Bishop

Serology Division

    Dr. B. S. Kline, *Chairman*

    Dr. C. G. Culbertson

    Dr. L. C. Todd

Fungi Division

    Dr. F. W. Shaw, *Chairman*

    Dr. J. C. Norris

Hematology Division

    Dr. Roy R. Kracke, *Chairman*

    Dr. F. J. Heck

    Dr. N. Rosenthal

The JOURNAL regrets to announce the death of Dr. C. C. Pflaum, formerly associated with Dr. Pinson Neal and recently pathologist for the St. Luke's Hospital in Duluth, Minnesota, and Dr. Charles Norris, Chief Medical Examiner of New York City.

The American Optical Company has recently purchased a substantial interest in the Spencer Lens Company. This will be of interest to the many users of microscopes and equipment manufactured by the Spencer Lens Company.

Last March Governor Lehman signed a bill granting authority to the courts of New York State to order blood tests in connection with cases involving paternity and interchange of babies. A similar bill was signed by Governor LaFollette of Wisconsin in August, 1935.

THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS ROSTER FOR 1935

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Dr. Foster M. Johns..................................President
Dr. R. A. Kilduffe..................................Vice-President
Dr. Roy R. Kracke..................................President-Elect
Dr. Alfred S. Giordano..................................Secretary-Treasurer

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Dr. F. H. Lamb, Chairman   Dr. K. Ikeda
Dr. L. W. Larson   Dr. W. M. Simpson
Dr. A. G. Foord   Dr. H. C. Sweany

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1923–4	Dr. Wm. Carpenter MacCarty	Rochester, Minnesota
1924–5	Dr. John A. Kolmer	Bala-Cynwyd, Pa.
1925–6	Dr. Frederic E. Sondern	New York, N. Y.
1926–7	Dr. Wm. G. Exton	New York, N. Y.
1927–8	Dr. A. H. Sanford	Rochester, Minnesota
1928–9	Dr. F. W. Hartman	Detroit, Michigan
1929–30	Dr. J. H. Black	Dallas, Texas
1930–1	Dr. K. M. Lynch	Charleston, S. C.
1931–2	Dr. H. J. Corper	Denver, Colorado
1932–3	Dr. Walter M. Simpson	Dayton, Ohio
1933–4	Dr. Alvin G. Foord	Pasadena, California
1934–5	Dr. Frederick H. Lamb	Davenport, Iowa

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BOOK REVIEWS

Clinical Diagnosis by Laboratory Methods. By James Campbell Todd and Arthur Hawley Sanford. 8th Ed. Philadelphia: W. B. Saunders Co., pp. 792, 1935. $6.00.

The many friends of this deservedly popular text will welcome this 8th edition and after reading it critically will not be disappointed in it.

The purpose of the book, as indicated in the title and preface, is carried out faithfully, so faithfully in fact that clinical pathologists who know too much may criticize parts of it on the grounds that they are too elementary but students, technicians and those physicians who only occasionally make laboratory tests will find these same parts invaluable.

Much new material has been added but by resetting and using a new page form the size has not been greatly increased. Among the many additions are the Addis count for urinary casts and cells, the urea clearance test for kidney function, a test for hemosiderin and one for amylase in the urine, and enlarged discussion of hemoglobin and the anemias, Sabin's technic for vital staining of blood, a description of the filament-non-filament leukocyte count, and a discussion of blood groups in relation to heredity and of the possibilities of the use of blood grouping in medico-legal cases where there is a question of paternity. The sections on serodiagnosis and bacteriology have likewise been revised and enlarged by the addition of the newer methods. The book is recommended without reservation to workers in the fields of clinical microscopy, chemistry, bacteriology, serology, and parasitology.

W. S. Thomas.

Laboratory Methods of the United States Army. Edited by James Stevens Simmons. Philadelphia: Lea & Febiger, pp. xx + 1091, 1935. $6.50.

It is not surprising that officers of the medical department of the United States Army should be able to write a creditable manual dealing with laboratory methods. It is gratifying that they have not only done that, but have by their combined efforts produced what may well be considered the finest thing of its kind yet published. Each section is written by an authority in the field and the whole is so carefully edited as to make it uniform in its high quality throughout. It is evident there is no second hand information which is so conspicuous in many other texts. Besides the usual chapters on technic, one finds some that are unique and deserve special mention: these are on water, sewage and industrial wastes, food and beverages, toxicologic methods, examination of shell fish, entomology, special veterinary methods, and statistical methods. The chapter on entomology is especially valuable and the best of its kind the reviewer has seen.

Throughout, the text is clearly worded, specific, brief yet comprehensive, and critical reading reveals no errors worth noting except that the importance of using calibrated hemocytometers is overstated; the figures of *Endomoeba histolytica* represent the nuclei as being too coarse in comparison with those of *Endamoeba coli*. It is significant and gratifying that the nomenclature of bacteria follows the Bergey manual.

The purchasers of this manual, and there should be many, will possess a text of undisputed authority, one that is comprehensive and contains a wealth of readily available, useful information. No laboratory will be complete without it.

Laboratory Diagnosis. By EDWIN E. OSGOOD. Philadelphia: P. Blakiston's Son & Co., pp. xx + 585, 1935. $6.00.

This edition, containing over 100 pages more than the first which appeared under the authorship of Osgood and Haskins, is a greatly improved book. Although adhering to the same general viewpoint, there are many changes in the text. The chief additions concern the technic and interpretation of the blood-urea clearance test, insulin coefficient, blood bromide determination, hormone tests, heterophil antigen tests, protein determinations,

and the forensic application of blood grouping. In addition, major changes have been made in the section on hematology where new work in the field has altered old conceptions. There have been added four new color plates and several new tables and drawings. All told, the book is more accurate, more useful, and altogether very satisfactory.

Clinical Parasitology and Tropical Medicine. BY DAMASO DE RIVAS. Philadelphia: Lea & Febiger, pp. 367, 1935. $5.00.

The aim of this book is to supply a text which will be more than an outline and less than an encyclopedia on modern progress in tropical and parasitic diseases. The major divisions deal with: (1) general considerations of etiology and pathology, environmental conditions, laboratory diagnosis, and treatment, (2) diseases due to protozoa, (3) diseases caused by metazoa, (4) bacterial diseases, (5) diseases of undetermined etiology, and (6) climatic diseases and animal poisons. While there is much useful information in the book, many subjects are superficially treated and some statements are extremely misleading. The most obvious shortcomings are noted in the therapeutic measures advocated, the author recommending his irrigation method for many diseases and omitting many useful methods; for example, he does not mention hexylresorcinol in the treatment of nematodes in spite of the excellent literature available. No reference is found to treparsol or carbarsome, no discussion of the use of arsenicals in the treatment of amebiasis, nor is calcium mentioned for treating trichiniasis. Many unsupported statements can be found, such as, for example, that *Endamoeba coli* probably are at least potentially pathogenic; that any amebas should be removed from the colon, and that snakes are identified by the shape of their heads, color of their bodies, and configuration of their tails. There are many unjustified omissions, as for example, discussion of mosquito species, eosinophilia in trichiniasis, and the skin test for the diagnosis of this and hydatid disease. All the recent extensive American and Russian work on Diphyllobothrium, and recent x-ray studies of sprue, are omitted.

It would be tedious to list all the minor mistakes in the book, but there is little excuse for the numerous errors in spelling, both of the names of well-known investigators and of parasites; for example, Diphyllobothrium is misspelled three times on page 21 and twice on page 22, and Kofoid is misspelled twice on page 351.

The book will require extensive revision before it will become a recognized authoritative source of information on the subject.

General Bacteriology. By EDWIN O. JORDAN. Philadelphia: W. B. Saunders Company, pp. 825, 1935. $6.00.

This edition contains some rewritten paragraphs scattered throughout the text and in addition a new chapter on bacterial variation which will give the elementary student a clear insight into the present status of this controversial field. The chapters receiving the most revision are those dealing with immunity, streptococci, Salmonella, Brucella, Rickettsia, and the viruses. Essentially, however, the book is much the same as in its earlier editions.

The author is still struggling with nomenclature and, unwilling to swallow the entire Bergey manual, finds himself choking on parts of it, errors in proof reading sometimes resulting in curious mixtures. The misleading paragraph on surgical technic should long ago have been deleted.

The book has been recognized as a standard text for years.

Food and Beverage Analyses. By MILTON A. BRIDGES. Philadelphia: Lea & Febiger, pp. 248, 1935. $3.50.

This volume fills an urgent need for authoritative data on the analysis of foods and beverages. After years of careful investigation, all the available data are assembled in this volume, which consists primarily of tables, including almost every known food both natural and canned. The vitamin content of foods is also indicated and a valuable bibliography concludes the text. The book is an essential one for hospitals, dietitians, and all those who must prescribe diets.

Free Medical Care (Socialized Medicine). By E. C. Buehler
  New York City: Noble and Noble, pp. iii + 360, 1935. $2.00.

This book was primarily intended to be used by high school debaters debating under the auspices of the University Extension Association.   The book is a resumé of the arguments for and against free medical care and consists of eighteen reprinted articles, forty-two pages of bibliography, thirty pages of "digested" matter and chapters dealing with various aspects of socialized medicine.   Not only will the volume prove interesting and helpful to the high school students engaged in these debates but may be read with profit by physicians.

Index photographed at the
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of the microfilm user.